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TRANSLATIONS FROM PRAKTICHESKIYE ZANYATIYA PO EPIZOOTOLOGII S MIKROBIOLOGIYEY

(Practical Exercises in Epizootology and Microbiology)

by I. A. Bakulov

- USSR -

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TRANSLATIONS FROM PRAKTICHESKIYE ZANYATIYA PO EPIZOOTOLOGII S MIKROBIOLOGIYEY

[Following are translations of selected articles from the Russian-language book by I. A. Bakulov entitled Prakticheskiye Zanyatiya po Epizootologii s Mikrobiologiyey (Practical Exercises in Epizootology and Microbiology), Moscow, 1962.]

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Exercise 7

Technique of Streaking Microbes on Nutrient Media and Cultivation Methods

Assignment. 1. To become acquainted with the technic of streaking microbes on simple nutrient media, subculture technic and technic of isolating pure cultures.

2. Independently to make cultures from pathological material on MIA [meat infusion agar], MIB [meat infusion bouillon] as well as subcultures from one medium to another.

3. To become acquainted with the incubator and methods of cultivating microbes.

Basic Equipment and Materials. The prepared nutrient media (MIA, MIB, MIG [meat infusion gelatin]) in test tubes, agar in flasks for pouring out into bacteriological plates, electric stoves, a water bath for melting the agar, an alcohol burner, bacteriological loops, Pasteur pipets, glass-marking pencils, spatulas, scalpels, forceps and trays. Agar which has been poured out beforehand into bacteriological plates, and an incubator. Organs from a dead animal, which died of a noncontagious disease. The bodies of experimentally-infected laboratory animals can be used. A culture of staphylococcus or colon bacillus in test tubes. A container with a disinfectant fluid.

Place of the Exercise. The laboratory of the technical school.

Method of Performing the Exercise. After a brief explanation, the instructor demonstrates the technic of culture from the organs of a dead animal body to simple nutrient media: MIA, MIB. It is necessary to show how to make the culture with a Pasteur pipet and bacteriological loop.

The instructor acquaints the students with the technic of subculture from one medium to another and with methods of isolating pure cultures (by means of a streak culture with a loop or with a glass spatula in bacteriological plates), with the methods of cultivating microbes, and explains the set-up of the incubator.

The students are divided into groups of three. They independently make the cultures on MIA and MIB from the organs of the dead body and subcultures of the colon bacillus or staphylococcus from one medium to another. Then, they melt the agar in the flasks, pour it out in a sterile manner into bacteriological plates, and on other bacteriological plates with medium which has been poured out beforehand they

make streak cultures of the colon bacillus (instead of the usual MIA the students can be given Endo agar or Bactoagar "G"). The cultures made by the students are marked and put into the incubator; they are studied at the next exercise.

Technic of Cultivating Microbes on Nutrient Media. For the purpose of obtaining microbe cultures from pathological material a small part of it is introduced into some nutrient medium with a bacteriological loop or Pasteur pipet. This process is called "culturing."

In order to make the culture, the bacteriological loop or Pasteur pipet is taken in the right hand between the thumb, index and middle fingers. Before making the culture the bacteriological loop or Pasteur pipet is flamed on a burner (the fine end of the pipet thereby is bent at a right angle). The part of the organ from which the culture is to be made is cauterized directly before making the culture with a heated metal spatula or is cut with a sterile scalpel (when the culture is made with a bacteriological loop).

Two test tubes containing nutrient media (MIB and MIA) are taken in the left hand. The test tubes are held in an inclined position between the thumb, index and middle fingers of the left hand, the tip of the pipet is broken off, and the pipet is rapidly passed through the flame of the burner; the organ is pierced in the cauterized spot, and a small quantity of material is sucked up into the capillary of the pipet. In making a culture from liver or spleen the pulp is sucked up by movement of the capillary forward and backward without removing the capillary from the organ and without piercing the organ with it. After this, both test tubes are opened simultaneously with the right hand (the cotton stoppers are held between the little finger and the palm of the right hand), the edges of the test tubes are flamed, the pipet with the material is inserted into a test tube containing bouillon and then a small quantity of bouillon is rapidly sucked up into the capillary and transferred into the test tube with the MIA slant. The edges of the test tube are flamed, after which the test tubes are covered with cotton stoppers which have also been passed through a flame. The pipet used is immediately put into a container with disinfectant solution, and the bacteriological loop is carefully flamed and put on a rack. The name of the organ from which the pathological material was taken, the number of the examination and date of the culture are marked on the test tubes with a special pencil.

Technic of Subcultures. For the purpose of subculturing the microbes on another medium the same technics are used as for making cultures. Two test tubes are taken in the right hand: a test tube con-

taining the culture and a test tube with fresh medium. Both test tubes are held with the thumb, index and middle fingers of the left hand in an inclined position. The MIA must be with the slant facing upward.

The culture is made with a loop or pipet. The loop is flamed, the Pasteur pipet is passed through a flame, the capillary is bent at a right angle and the tip is broken off immediately for subculture.

Both test tubes are opened simultaneously on the burner flame with the right hand, holding the cotton stoppers between the little finger and palm of the right hand (Fig 28). The exposed edges of the test tubes are flamed. With a loop or pipet which has been passed through a burner flame a small quantity of material is taken up from the test tube containing the culture (the loop must first be allowed to cool within the test tube) and rapidly transferred with the loop to the test tube containing fresh medium.

Fig 28. Subculture of Microbial Culture om One Medium to Another: a. Opening test tube; b. Flaming loop and edges of the test tube; c. Taking of culture and subculture.

Cultures on MIA are made by means of a zigzag streaking of the material on the surface of the medium.

The culture on meat infusion gelatin is made by means of puncturing the central part of the medium with a platinum needle (a straightened-out bacteriological loop) containing the material, watching to see that the needle enter the medium as deeply as possible (the loop holder should not be allowed to come in contact with the medium). The test tube containing MIG is thereby held upside down (Fig 29).

Fig 29. Making Microbial Culture by Puncturing Meat Infusion Gelatin.

After the subculture has been made the edges of the test tubes are flamed, the test tubes are closed with stoppers which have been passed through a flame. The bacteriological loop is flamed and put in the rack; the pipet used is immediately put in a container with disinfectant.

The test tube containing the medium on which the subculture has been made is immediately marked.

Isolation of Pure Cultures. In a bacteriological examination a study is made of microbes in pure cultures, that is, without an admixture of microbes of other species.

For the purpose of isolating pure cultures usually the differential cultivation method for the microbial emulsion is used on the surface of a solid nutrient medium in culture plates.

Use is also made of the method of serial dilution of the material being investigated in liquid and solid fused media as well as the biological method for obtaining pure cultures of pathogens of contagious diseases by means of infecting laboratory animals.

Before making the differential culture on solid media the nutrient medium -- MIA (ready-made agar in the flasks is heated in a boiling water bath and then poured out in culture plates in a sterile manner) -- is poured out into plates. After the agar cools the plates are placed in an incubator for 24 hours in the upside-down position for the purpose of checking the medium for sterility.

In the case of culture on a plate the test tube with the microbe emulsion is taken in the left hand and held between the middle (at the top), index and ring fingers (Fig 30) in an inclined position.

Fig 30. Subculture of Microbe Culture on Bacterial Plate: a. Taking the material; b. Cultivation on bacterial plate.

After opening the test tube over a flame a small quantity of culture material is taken up in a loop heated to incandescence or a pipet. The cover of the plate is slightly raised and the material is spread out over the surface of the nutrient medium. In the case of making the culture with a pipet a spatula is first made from it (by means of bending it over the flame of a burner) and the culture material is rubbed in with this spatula.

Then, with the same loop or spatula (without flaming them!) two or three more plates are inoculated. Therein lies the nature of the differential method of cultivation.

After this, the loop is flamed and put in a rack. The inoculated plates are marked and allowed to stand in an incubator for 24-48 hours in the inverted position.

The separate colonies which grow out on the plates are studied by means of a magnifying glass or by using the low power of a microscope. The necessary colonies are marked with a pencil, and then material is taken with a loop from these colonies for subculture on nutrient media in test tubes. For the purpose of checking the purity of the culture obtained material may be taken from a separate colony, emulsified in sterile physiological saline and again streaked on bacterial plates.

After obtaining a pure microbe culture the study of it may be begun.

Methods of Cultivating Microbes. For the purpose of growing out the majority of microbes a temperature of 36-37° is necessary. For this, the inoculated nutrient media are placed in an incubator (Fig 31), which is a cabinet supplied with a heating apparatus, an adaptation for maintaining a constant temperature and a thermometer, the readings of which are recorded daily in a control sheet for the operation of the instrument.

There are several kinds of incubators. At present, the most commonly used is the electric incubator. In the incubator a constant temperature is maintained by a heat regulator, which operates automatically, now turning off, now turning on the source of heat.

The cultures are kept in the incubator until the appearance of culture growth (one-30 days), after which the cultures obtained are studied. Cultures on gelatin media are usually grown out at room temperature.

Fig 31. Modern thermostats

Exercise 8

A Study of Cultures Made Independently by the Students at the Previous Exercise

Assignment. 1. To study and describe the nature of growth of the culture on nutrient media.

2. To prepare smears from the culture and stain them by the Gram method.

3. To study the cultures for mobility.

Basic Equipment and Materials. Cultures made by the students at the previous exercise, glass slides and cover glasses, glasses with wells, a set of stains for staining by the Gram method, an alcohol burner, loops, physiological saline in test tubes, microscopes, seeder oil and vaseline.

Place of the Exercise. The laboratory of the technical school.

Method of Performing the Exercise. The instructor briefly describes the aim and methods of studying pure cultures, demonstrates the methods of investigating microbes for mobility. The students independently evaluate and describe the nature of growth in the cultures from the organs of the cadaver and then set about studying the pure culture of the colon bacillus, the subculture of which was made at the previous exercise.

The instructor directs attention to the nature of growth of the colon bacillus on solid (colonies) and liquid media. The students independently prepare smears from the cultures, stain them by the Gram method, examine them under a microscope and draw a picture of them. In conclusion, the students examine the cultures for mobility by the method of hanging or thin drops.

Study of Pure Culture. After isolation of the pure culture the study of it is begun. It is begun with a description of the nature of the growth of the microbe on liquid and solid media. Study of the majority of pure cultures is performed after 24 hours as a rule.

Evaluation of the growth on liquid media amounts to determination of the degree and nature of turbidification, the nature of the sediment, the presence or absence of a membrane or a ring next to the wall of the tube.

For example, the colon bacillus after 24 hours of growth on MIB produces a uniform intense turbidification; on the bottom of the test tube a sediment forms which comes up in flakes when shaken. A thin film

forms on the surface of the bouillon.

In studying growth on solid media attention is paid to the nature, size, shape, color and transparency of the colonies. The microbe colonies on solid media may be of different sizes and shapes. Up to one millimeter in diameter of the colonies are considered punctate; one-two millimeters, small; two-four millimeters, medium; more than four millimeters, large. Attention is directed to the edges of the colonies, which may be smooth or rough (wavy, fimbriate, like locks of hair). The surface of the colonies is also different: smooth, rugose or streaked. Colonies are also differentiated by their transparency (clear, opaque, dull) and by their consistency (mucoid).

For example, the colon bacillus forms succulent, mucoid, flat, gray-white medium-sized opaque colonies with smooth borders on MIA in the first 24 hours of growth.

Then the cultures are examined under the microscope, for which smears are prepared and stained by the Gram method or other methods.

The next stage is investigation of the culture for mobility. For this purpose a culture is used which is no older than 24 hours. The investigation for mobility is accomplished by means of a hanging drop or a thin drop.

Preparation of the Hanging Drop. A special glass slide with a well is used (Fig 32) and carefully wiped off with dry gauze. Around the well a thin layer of vaseline is applied. A drop of the culture is applied to a prepared (defatted) cover glass. Before taking the drop from the culture the loop should be allowed to cool well. The drop is not spread out on the cover glass but applied in one spot. After this, the cover glass is attached to the glass slide with the well, for which purpose the glass slide with the hollow is turned over and the place where the vaseline has been applied is let down on the cover glass so that the drops of culture are in the center of the well. Then the prepared preparation is turned back over so that the cover glass is on top, and the drop is examined under the microscope.

Fig 32. Investigation of Microbes for Mobility: a. Glass with well; b. Hanging drop.

In the investigation of cultures grown out on solid media for mobility a drop of physiological saline is first applied to the cover glass, and the culture taken from agar with a loop is emulsified in it.

At the end of the examination the preparation is removed from the

microscope stage, the cover glass is carefully moved so that it project over the edge of the glass slide with the well, it is taken with a forceps and put into the container with disinfectant solution.

Preparation of a Thin Drop. A small drop of the culture to be investigated is applied to a defatted glass slide and covered with a cover glass. The preparations made are examined in the following way. The opening of the microscope diaphragm is reduced, or the condenser is let down somewhat. Using an 8x objective, the edges of the drop are sought, and then, without moving the preparation, it is studied with the 40x objective. If necessary the preparation can be investigated in an oil immersion system, for which a drop of cedar oil is applied to the cover glass and the objective is let down on it carefully under visual control.

The nature and degree of mobility of different microbes may be different. Mobility may be good, moderately or poorly expressed. In evaluating mobility it is necessary to study the paths of several individual cells. In a pure culture there may be nonmotile cells (older or dead) along with the motile cells. Sometimes the microbe cells move as though they were rotating.

In order to differentiate poor motility of microbe cells from molecular (Brownian) movement which is characteristic of fixed microscopic particles, the following may be done. After making the observation of the microbes in a hanging drop and keeping in mind the nature of their movement, the cover glass is cautiously removed, the residual vaseline is removed from it, and it is heated on a burner flame until the drop dries, thereby assuming a gray-white color. Such a heat-killed culture is emulsified in a drop of sterile physiological saline applied to the dried culture and again studied under the microscope, thereby comparing the nature of movement of the microbes before and after heating. If the nature of movement of the microbes does not change the culture being studied belongs to the group of nonmotile bacteria.

Further study of the pure culture is made by means of subcultures of it on special nutrient media (color series, selective media -- Endo-agar and others) for the study of biochemical properties, the capacity of pigment formation, hemolytic properties and others.

When necessary, serological studies are made of the culture isolated and laboratory animals are also infected.

At the end of the investigation there should be clear-cut data as to the morphologic, cultural, biochemical and serological properties and pathogenicity of the culture being studied, on the basis of which a given

species of microorganisms is determined, utilizing for this purpose special tables or classifiers of microbes.

Exercise 9

Rules for Working with Laboratory Animals

Assignment. 1. To prepare instruments and material for infecting laboratory animals.

2. To study technics of fixation of laboratory animals; to take the temperatures of rabbits and guinea pigs.

3. To infect rabbits, guinea pigs, white mice and pigeons by different methods.

4. To dissect the bodies of rabbits, guinea pigs, white mice and pigeons.

5. To become acquainted with the method of taking blood from laboratory animals.

Basic Equipment and Materials. Laboratory animals: rabbits, guinea pigs, white mice and pigeons. The dead bodies of killed laboratory animals: rabbits, guinea pigs, white mice and pigeons. Ether or chloroform, a glass belljar. One-two-cc syringes with needles, sterile packs prepared beforehand containing cotton for expelling the air from a syringe during injection, sterilizers, forceps, scissors, scalpels, cuvettes filled with wax or panels for fixation of the bodies, small basins or trays, pins for fixation of the bodies, an electric heater or Primus (Swedish stove), alcohol, cotton, hemostatic forceps (Pean or Kocher), two percent carbolic acid solution. An alcohol burner. Empty cages or glass jars for the infected animals. Colon bacillus cultures for infection. Sterile mortars and pestles for preparation of emulsions, physiological saline solution in flasks, test tubes for taking blood (measuring 14x65 millimeters). A paper for labels. Paint for marking the animals.

Place of the Exercise. The laboratory of the technical school.

Method of Performing the Exercise. The instructor briefly acquaints the students with work on laboratory animals for the diagnosis of infectious diseases; demonstrates methods of fixation of laboratory animals; explains how to prepare the instrument and material correctly for the infection; shows different methods of infecting animals.

The students are divided into groups of four, which independently prepare the instruments and materials for the infection (emulsions, cultures), take the temperatures of the rabbits and guinea pigs, infect laboratory animals by different methods (subcutaneously, intramuscularly, intraperitoneally and intravenously). Instead of the culture the

student may be given a sterile physiological saline solution. After the infection the students mark the animals, place them in empty cages, mark the cages or jars, and sterilize the instrument which has been used.

The instructor shows the rules for dissecting the dead bodies of laboratory animals. The students independently dissect the bodies of several laboratory animals (several animals can be killed with ether right at the exercises); the pathological changes observed on autopsy are described.

In conclusion, the instructor acquaints the students with the method of taking blood from rabbits and guinea pigs (from the auricular vein and heart).

Work with Laboratory Animals. Laboratory animals are used for determining the pathogenicity and virulence of the microorganism being studied, for the isolation of pure cultures of pathogenic microbes from mixtures with saprophytes. In using animals of one species or another consideration is given to their degree of sensitivity to pathogens of certain infectious diseases (for example, the pigeon is very sensitive to infection with swine erysipelas).

The animals designed for infection should be healthy, should be in a good state of nutrition, have smooth shiny fur and a normal body temperature.

The body temperatures of guinea pigs and rabbits are taken with an ordinary thermometer. The depth to which it is inserted into the rectum has an influence on the thermometer readings; therefore, in order to obtain comparable results it is necessary to insert the thermometer to the same depth. This is achieved by setting a rubber ring a certain distance from the end of the thermometer. Subsequent body temperature measurements should be made at the same time.

Before infecting the animals they are marked, weighed, the sex is determined (if necessary). They are marked by means of metal tags or branding irons. Usually, in the laboratory animals are marked by painting various parts of the body with aniline dyes (for this purpose it is best to use Ziehl's carbol fuchsin; the fuchsin marking is readily seen and stays on for one-two months).

The instruments for the infection (one-two-cc syringes, needles, forceps and others) are boiled in distilled water for 10-12 minutes. Special attention should be paid to the fit of the syringe plungers as well as to the fit of the needles on the syringes. Faulty syringes and poorly fitting needles should not be used, because this can lead to infection of man.

At the same time, everything necessary for the infection is prepared: cotton, alcohol, labels on the cages for the infected animals, small packs containing cotton sterilized beforehand.

The packs serve for expelling the air from the syringe in infection of the animals. They are made from a piece of paper measuring 7x10 centimeters; a small piece of cotton is wrapped in the pack in the same way in which drug powders are packed. The packs made in this way are sterilized in an autoclave. Before infection such a pack is put on the needle; the syringe (with the culture or emulsion) is kept vertically (with the needle up) and by a slight movement of the plunger the air is removed from the barrel without the risk of infecting the surrounding objects with infectious material.

After the work, the instruments are again boiled in distilled water for 20-30 minutes (depending on the material investigated).

For the infection of laboratory animals a culture of microbes or an emulsion made of material sent in for investigation is used. Usually, laboratory animals are infected with a 24-hour bouillon or agar culture. A washing is made from the agar culture, for which purpose several cubic centimeters of sterile physiological saline solution are added to it in a sterile manner and, rotating the test tube between the palms, the microbe culture is washed off the agar. Separate colonies are removed from the agar with a loop and emulsified in a small quantity of sterile physiological saline.

For the purpose of preparing the emulsion mortars and pestles sterilized beforehand are used. Pieces of the most infected parts of the organ or tissue are taken with sterile instruments (scissors, scalpels, forceps), the material taken is ground up, sterile physiological saline is added, and the material is carefully ground up in the mortar with the pestle (when necessary, sand heated to incandescence is added). For the purpose of obtaining emulsion 10 parts of physiological saline are used for one part of material being investigated. The emulsion is injected into the animal immediately or after preliminary filtration through a cotton-gauze filter. The serum, blood, or exudates are injected into the animals without preliminary preparation.

Methods of Fixation of Laboratory Animals. Rabbits are taken out of their cages by the skin of the back and are placed on a table. The investigator places his hand under the abdomen and holds both hind extremities (as high as possible), and then the rabbits are lifted up on the hind extremities and both anterior extremities are held with the other hand. After this, the rabbits are removed from the table and stretched out to their full length (Fig 33, a).

Fig 33. Methods of Fixation of Laboratory Animals: a. Fixation of rabbits; b. Fixation of guinea pigs; c. Fixation of white mice.

After injecting the material intravenously or in the case of taking blood from the auricular vein of the rabbit it is most convenient to wrap the animal in a piece of thick material, leaving only the head free.

Guinea pigs are taken out of the cage with one hand, grasping the trunk behind the fore-extremities. They are held in two hands: the hind extremities are held together with one; the anterior extremities and head are held with the other (Fig 33, b).

White mice are taken out of the cage with a forceps or are taken by the tail and lifted up quickly. Then, the mouse is let down on the person's gown or on a table, grasped by a skin fold in the area of the nape of the neck with a rapid movement, and the mouse is held extending it with two hands. All the manipulations should be done quickly in order to avoid bites. If it is impossible to grasp the mouse by the skin of the back of the neck several rotary movements may be made first, holding the mouse by the tail.

In working without an assistant the mouse is held by two methods.

1. The mouse is grasped with hemostats (Pean or Kocher) by the skin fold in the area of the back of the neck and the hemostat is attached to some object by the rings used for the fingers (for example, to the rod of a burette stand). With one hand the mouse is held by the tail; with the other the injection is made (Fig 33, c).

2. The mouse is grasped quickly with the thumb and index fingers by the skin fold in the area of the back of the neck, rotated with its abdomen up, thereby grasping the tail and the left hind extremity with the little finger and ring finger of the same hand. The injection is made with the other hand.

Pigeons are held with one hand, grasping the trunk and simultaneously pressing both wings together and both extremities against the body.

In holding laboratory animals caution should be observed in order to protect oneself and not cause harm to the animal by rough fixation.

Methods of Infection of Laboratory Animals. Usually, laboratory animals are infected subcutaneously, intramuscularly, intraperitoneally or intravenously. Sometimes, recourse is had to intradermal or intracerebral methods of infection; the animals are infected through the digestive tract as well as by means of application of material to the conjunctiva or to the scarified skin. At the injection site the fur is shaved off, the skin is treated with alcohol or two percent phenol solution.

The material is injected into rabbits subcutaneously in the area of the back or abdomen; guinea pigs, in the region of the abdomen; white mice, in the region of the back at the root of the tail. In order to prevent leakage of the material, the puncture site is held with a clamp and compressed; a piece of cotton moistened with collodion may be attached to it. The volume of material injected is usually 0.5-two cc for the rabbit; 0.5-one cc for the guinea pig; 0.2-0.5 cc for the white mouse.

In the case of intramuscular infection of rabbits, guinea pigs and white mice the material is injected into the depths of the muscles, on the inside in the thigh region; in pigeons, into the pectoral muscle after first pulling out the feathers at the injection site. In the case of infection of pigeons the needle should be directed toward the head rather than toward the trunk, because in the latter case injury to the peritoneum is possible.

In the intraperitoneal method of infection the animal is held with the head down (to avoid wounding the intestine). For the injection short needles with dull edges are used. The material is injected into the left lower third of the abdomen. First, the needle is directed horizontally, simultaneously piercing skin, musculature and peritoneum. Then, the syringe is shifted to the vertical position and the material is injected slowly.

Intravenous method of infection is mostly used in rabbits. The injection is made into the marginal vein of the ear. At the base of the ear the vessel is compressed with the fingers for better blood-filling of it. The ear may be first wiped off with xylol or flicked with the fingers. This causes a filling of the vessels with blood and facilitates injection of the needle into the vein. With the left hand the ear of the rabbit is taken so that the index finger is down and the thumb presses the ear upward. For convenience the ear is bent slightly on the index finger. The syringe furnished with a fine needle is taken with the right hand, and the needle is injected intravenously along the course of the blood stream (in the direction toward the head) (Fig 34). With proper injection of the needle into the vein the material enters freely with slight pressure on the plunger. In an incorrect position of the needle a swelling forms at the injection site; in this case the injection is repeated nearer the base of the ear.

Fig 34. Intravenous Infection of the Rabbit: a -- marginal vein of the ear.

The material should be injected slowly intravenously. The dose for the rabbit amounts to one-five cc usually. At the puncture site a piece of cotton is applied.

After infection of the animals they are put in separate cages (mice may be kept in glass jars closed over with a metal screen). Labels indicating the number of the cage or jar, the last name of the worker responsible for the experiment (or student), date, number and species of animals, with what the animals were infected, modes of infection, dose injected, how the animals were marked are put on the cages or jars.

The infected animals are kept in separate rooms isolated from healthy ones; separate personnel takes care of the animals.

Dissection of the Bodies of Laboratory Animals. Dead laboratory animals are dissected and subjected to pathological and bacteriological studies. Sometimes, it is necessary to resort to sacrificing the animals. For this purpose they may be placed under a glass belljar, putting cotton moistened in ether or chloroform into it.

Rabbits may be killed by an electric current. For this purpose, an electric cord with an ordinary two-pin plug at one end and with injection needles attached to both wires at the other end is used. The needles are injected subcutaneously: one, in the area of the nape of the neck; the other, in the lumbar region (or tail); after this, the current is turned on.

The body should be dissected immediately after death of the animal. The autopsy is performed on tables covered with galvanized iron or in metal trays. The bodies are fastened with pins, snap fasteners or nails to a wooden or cork panel, which is then put into the tray. It is expedient to pour fused wax over the tray. After this, as the wax solidifies, the body is fixed by means of pins. After the dissection the wax is decontaminated by means of remelting.

Before the dissection the instruments are prepared (scalpels, scissors and forceps); everything necessary for making the cultures and preparing the smears is also made ready: glass slides, pipets and loops, spatulas for cauterization, an alcohol burner, nutrient media, a glass marking pencil, a container with disinfectant solution, and a notebook for notes.

For disinfection of the integument and for the destruction of skin parasites the bodies are immersed in two percent phenol solution for several minutes. The fur may be moistened in alcohol and then burned off.

The dissection is begun with an incision along the linea alba, from

the pubis to the neck. The skin is dissected away on both sides. The chest is incised with scissors, first cutting the diaphragm and then the ribs on both sides at the sites of their attachment to the sternum; the latter is separated from the body. For better examination of the abdominal organs the abdominal wall should be cut perpendicularly to the first incision on both sides to the lumbar vertebrae. When necessary, the cranial cavity is opened, and the brain is investigated.

After a cursory examination of the organs cultures are made from the internal organs and heart blood (see Exercise 7), smear-impressions are made from the organs (see Exercise 3) [the organ is touched to the glass slide several times], and after this a careful autopsy is performed. The results of examination of the body and of pathological examination are recorded on a laboratory examination form.

After the work the instruments are sterilized by boiling: the body of the animal is burned. The table and trays are poured over with three percent phenol solution for two hours or with five percent lysol solution for the same time. The tables may be moistened with alcohol and then flamed. The cage in which the laboratory animal died is disinfected; the litter and food remains are burned.

The Taking of Blood from Laboratory Animals. Blood from laboratory animals (usually from rabbits or guinea pigs) is taken for examination as well as for the preparation of complement or sera necessary for the performance of serologic tests. In guinea pigs and rabbits the blood may be taken from the auricular vein or from the heart.

Blood is taken from the auricular veins by means of incision or puncture of the blood vessel. It is convenient to take blood from the marginal vein of the ear. The fur at the site of the operation is shaved off or plucked out.

For better filling of the blood vessel of the ear the latter is wiped with cotton moistened with xylol. The blood vessel is compressed at the base of the ear, and a transverse incision into the blood vessel is made with a sharp scalpel or razor blade. The first drop of blood is removed with sterile cotton, and then a test tube (14x65 millimeters) is brought up to the site of the incision and filled with blood (Fig 35, a), whereby the blood should come out along the wall of the test tube as a steady stream.

Fig 35. The Taking of Blood: a. From the auricular vein of the rabbit; b. From the heart of the guinea pig.

A large quantity of blood (in a more sterile manner) can be taken

from the rabbit or guinea pig by means of a cardiac puncture. The assistant holds the guinea pig on the table in a position on its back, slightly tilting it on itself with the left side toward the operator. With the index finger of the left hand the operator finds the point where the cardiac impulse is felt most strongly. This point is usually in the middle of the chest, somewhat to the left (0.5-one centimeter) of the midline. For the purpose of obtaining blood a five-10-cc syringe with a thick needle two centimeters long is used. The needle point should be dulled first. The fur is shaved off at the puncture site; the puncture site is disinfected.

The chest wall is pierced by holding the syringe in the right hand in a somewhat inclined position in a direction from front to back (Fig 35, b). During the puncture the index finger should be held on the plunger and an attempt should be made to move it back slightly so that a negative pressure is created in the barrel (rarefaction of air). With correct entrance into the heart the plunger rises slightly, and the barrel is filled with blood.

From an adult guinea pig five-six cc's of blood can be taken at one time; from a rabbit, up to 10 cc's of blood. After this, the animal is immediately given a subcutaneous injection of physiological saline solution corresponding to the amount of blood taken heated to body temperature.

Blood can be taken again from the same animal no sooner then 10-15 days.

Exercise 12

Biologicals Used in Veterinary Practice

Assignment. 1. To become acquainted with different types of biologicals.

2. To study the rules of rejecting, storage, transportation of biologicals and disinfecting the containers for the biologicals.

3. Independently to determine the suitability of biologicals.

4. To calculate the requirement for biologicals.

Basic Equipment and Materials. Biologicals of different types for demonstration: vaccines, sera, bacteriophage, toxoids, antiviruses, allergic preparations, preparations for laboratory diagnosis. Among these preparations there should be some suitable for use and some which are unsuitable (which have gone beyond their service period, with damaged labels, faulty sealing, with an altered macroscopic appearance).

Veterinary legislation or various instructions on the use of biologicals (for the purpose of solving problems).

Place of the Exercise. A lecture-room of the technical school.

Methods of Performing the Exercise. The instructor gives a brief characterization of biologicals used in veterinary practice, directs attention to the classification of biologicals, acquaints the students with the rules of discarding, storing, transportation of biologicals.

Special attention should be paid to the disinfection of containers of the biologicals.

For each student or group of three persons several small bottles of biologicals are prepared for independent evaluation of their suitability.

In conclusion, the instructor gives an assignment of calculating the amount of biologicals to be used for a certain infectious disease. The students are given the appropriate instructions on the use of the biologicals. At the end of the exercise several students are questioned in order to check whether they have solved the problem correctly.

The biological industry of the Soviet Union produces more than 80 biologicals for veterinary purposes. They are prepared at biologicals plants and at bacteriological institutes according to a method worked out for the preparation of each type. The State Scientific Control Institute of Veterinary Preparations is responsible for checking on the observance of these methods and the quality of the biologicals. Before

being released the preparations are checked for sterility, harmlessness and activity.

All biologicals used in veterinary practice can be divided into three groups (Fig 42).

Fig 42. Classification of Biologicals Used in Veterinary Practice. 1. Vaccines, toxoids, bacteriophages and antiviruses. 2. Sera. 3. Diagnostic media.

The vaccines are divided into three groups: a) vaccines made of living nonattenuated cultures; b) vaccines made of living attenuated cultures; c) vaccines prepared from inactivated (killed) cultures.

Vaccines Made from Living Nonattenuated Cultures. They are used for active immunization of animals against certain infectious diseases. Such immunization in a number of cases is associated with the need for quarantining a farm, because the inoculated animals excrete the infectious principle into the environment for a certain time.

For easier recovery from a sickness preparations containing living nonattenuated cultures are injected into the animal simultaneously with immune sera (simultaneous injection).

Among the preparations containing living nonattenuated cultures are the following: ovinia, used for prophylaxis of sheep pox; embryo vaccine of the State Scientific Control Institute, against bird pox and avian diphtheria; a peripneumonia culture, against epidemic pneumonia in cattle.

Vaccines Made of Living Attenuated Cultures. After injection of vaccines prepared from living attenuated cultures the organism readily recovers, thereby acquiring immunity which in its nature is very similar to immunity occurring as the result of the natural disease. Some of these vaccines are also used simultaneously with immune sera (for example, an avirulent dry lapinized vaccine virus against hog cholera).

Among the vaccines made of living attenuated cultures are: Tsenkovskiy's first and second anthrax vaccines; the STI (Sanitary Technical Institute) anthrax vaccine; the GNKI (State Scientific Control Institute of Veterinary Biologicals) aluminum-hydroxide vaccine against anthrax; long-acting vaccine against swine erysipelas; antirabies phenolized vaccine; dry brucellosis vaccine from strain No 19; avirulent dry lapinized virus vaccine against hog cholera (ASV); a virus vaccine against fowl plague; a dry virus vaccine against Newcastle disease, made of the B_1 strain and others.

Vaccines Derived from Inactivated (Killed) Cultures. Inactivation of cultures used for the preparation of vaccines is achieved by addition of formalin or other chemical agents to them.

Among the vaccines made of killed cultures are the following: aluminum-hydroxide formol vaccine against bacillary swine erysipelas; concentrated formol alum vaccine against calf paratyphoid; a formol vaccine for paratyphoid in young pigs; a formol vaccine against diplococcal septicemia; a formol vaccine against black quarter in cattle and sheep; a formol vaccine for lamb dysentery; a semiliquid formol vaccine for bradzot in sheep; bivalent formol-alum vaccine against infectious enterotoxemia and bradzot in sheep; long-acting formol-aluminum-hydroxide vaccine for cattle plague; aluminum-hydroxide-formol vaccine for sheep pox; aluminum-hydroxide-formol-embryonic vaccine for fowl plague; quinosol vaccine for leptospirosis; glycerinated crystal-violet vaccine for hog cholera; aluminum-hydroxide-formol vaccine against Aujeszky's disease; precipitated formol vaccine against pasteurellosis of long-horned cattle, sheep and swine and others.

Toxoids. In veterinary practice alum-precipitated tetanus toxoid is used for active immunization against tetanus.

Bacteriophages. a) Gärtner [Salmonella enteritidis] phage used for calf paratyphoid; b) Suipestifer phage used for paratyphoid of young pigs; c) Coli phage, use for colibacillosis; d) Coli-Gärtner phage used for mixed infection (colibacillosis and paratyphoid).

Antiviruses. The strangles antivirus is used for therapeutic purposes in strangles in horses.

2. Immune sera are prepared by means of hyperimmunization of the animals with bacterial and virus antigens. It is used for prophylactic and therapeutic purposes. In veterinary practice sera are used against the following infectious diseases: anthrax, swine erysipelas, colibacillosis and calf paratyphoid, paratyphoid in young pigs, lamb dysentery, tetanus, hemorrhagic septicemia, chicken cholera, diplococcal infections, leptospirosis, hog cholera, sheep pox, Aujeszky's disease, cattle plague and others.

3. Diagnostic preparations used in veterinary practice are divided into: a) preparations used for allergic diagnosis; b) preparations used for serologic diagnosis.

Preparations Used for Allergic Diagnosis. In this group are: mallein for the diagnosis of glanders; tuberculin for the diagnosis of cattle tuberculosis; tuberculin for diagnosis of avian tuberculosis and paratuberculosis in cattle; abortin for the diagnosis of brucellosis in

young cattle; brucellizat for the diagnosis of brucellosis in sheep and goats; brucellogidrolizat [hydrolysate] for the diagnosis of brucellosis in sheep, goats and swine; an allergen for the diagnosis of epizootic lymphangitis.

Preparations Used for Serologic Diagnosis. Precipitating anthrax serum, anthrax antigen (precipitinogen), brucellosis agglutinating serum, brucellosis antigen for agglutination test and complement fixation test, agglutinating paratyphoid sera, paratyphoid antigens, positive glanders serum, glanders antigen, hemolysin, positive epidemic peripneumonitis serum, epidemic peripneumonitis antigen and others.

In this group is the white bacillary diarrhea antigen used for the diagnosis of pullorosis and fowl typhoid.

Rules for the Evaluation and for Rejecting Biologicals

Preparations released for veterinary practice should correspond to certain conditions and a certain macroscopic appearance. All bottles containing biologicals should be tightly closed, poured over with sealing wax and sealed (Fig 43, a).

Fig 43. Bottles with Biologicals: a) bottle with a stopper poured over with sealing wax; b) bottle with a stopper fastened with a metal holder.

At the present time biologicals are also released in bottles closed with rubber stoppers with metal holders; these stoppers are not poured over with sealing wax and are not sealed (Fig 43, b).

On every bottle there should be a label of a standard model, on which the type of biological, its series, for what animals it is used, the doses and method of use, date of preparation, what institution prepared it, the longevity and the state inspection number are indicated.

Preparations in ampules are put in boxes with labels; the ampules are appropriately marked (Fig 44, 45).

Fig 44. Ampules Containing Biologicals.

Fig 45. Labels on Boxes Containing Ampules [the upper label is for brucellizat in which all the data mentioned in the last paragraph are indicated; the same applies to the second label which is dry virus vaccine against fowl plague].

In examining biologicals before use attention should be paid first of all to the service period of the given preparation. Preparations which have become outdated are discarded. Then, the sealing and integrity of the bottle are checked. The bottles should be without cracks, with an intact sealing wax seal; the fluid should not be sucked up through the stopper.

Some biologicals have a precipitate, which on shaking should be broken up into a uniform turbidity. If on shaking clumps remain which are not broken up, the preparation in these bottles is discarded. Biologicals in bottles in which mold or impurities are found are also discarded.

Biologicals should not be used if there is no label on the bottle or if the state inspection number is absent from the label. Biologicals are discarded when they are frozen, in case they have a putrefactive odor, change in the established consistency or color.

Transportation and Storage of Biologicals

Transportation of large batches of biologicals by railroad over great distances is carried out in special railroad cars with the appropriate temperature. Small quantities of biologicals are shipped by passenger train, by mail or express messenger. The methods of transporting biologicals depend on their types.

Biologicals made of living nonattenuated cultures as well as preparations with a short longevity are sent by express messenger; it is forbidden to send such preparations by mail or in baggage.

During transportation the preparations must be protected against freezing and the effect of high temperatures; damage to the bottles should not be permitted.

On storing the biologicals conditions should be observed which do not alter the macroscopic appearance or specific properties of the preparation for the established longevity. Freezing, high temperature, increased humidity and direct sunlight can change the quality of the biological. Biologicals should be stored in dry, dark, cool rooms with a uniform temperature from +2 to +15°. Unstable biologicals are kept under constant temperature conditions from +2° to +8° (best in refrigerators). Under conditions of a veterinary hospital or veterinary station the biologicals may be kept in cellars. A special place is outfitted (shelf, drawer, special cabinet) for keeping biologicals of each type. It is forbidden to keep good and rejected preparations together. Biologicals should not be kept with drugs.

The room in which the biologicals are kept should be closed and locked; the key is kept by a responsible person. A strict record of the intake and consumption of biologicals is kept in a special book. The utilization of unutilized biologicals from previously opened bottles is forbidden. The bottles for the biologicals are disinfected by means of autoclaving or boiling. Special attention should be given to disinfecting the bottles of biologicals which contain living cultures.

Exemplary Problem for Assignment No 12

Cases of anthrax among cattle have been registered in a farm. In this farm the herd of animals has not been inoculated previously against anthrax. The following are on the farm: 674 head of cattle including: 169 cows; 45 heifers; 209 young over a year of age; 117 young under two months of age; 134 young [calves] from three months to one year of age. There are 19 head of horses over two years of age on the farm.

For the personal use of workers on the farms there were 52 head of cattle including: 23 cows, 18 calves under two months; 11 calves from three months to one year of age; as well as 10 head of goats under two months of age; six head of goats from three months to one year of age and 13 head over one year of age.

It is necessary to calculate the required quantity of biologicals for inoculation of the herd against anthrax and make up a statement for the biologicals.

Example of a Solution

For inoculation of this herd it is best to use aluminum-hydroxide-precipitated GNKI vaccine against anthrax. Agricultural animals of all species may be inoculated, with the exception of the young of these animals under the age of two months. Animals are inoculated with this vaccine once; the doses depend on the age.

Calculation of the vaccine requirements is made in the following way:

Cattle over one year of age	446x1. 0=446 cc
Cattle from three months to one year. . .	145x0. 5=72. 5 cc
Horses over three years of age	19x0. 75=14. 25 cc
Goats over one year of age	13x0. 2 = 2. 6 cc
Goats from three months to one year. . .	6x0. 1 = 0. 6 cc
Total vaccine required	535. 95 cc

Young agricultural animals under the age of two months are not inoculated with the aluminum-hydroxide-precipitated GNKI vaccine against anthrax; when necessary, it is given passively (as serum). Calculation of the serum requirement is made in the following way:

Young cattle under the age of two months . . . . 135x10=1350 cc
Goats under the age of two months . . . . . 10x10=100 cc
Total serum required . . . . . 1450 cc

Note. In making up the statement the student should be shown the increase in the quantity of the preparations required over set standards (within limits of 10 percent for "pouring").

Similar problems may be made up by the instructor for other infectious diseases.

Exercise 14

Organization of Mass Treatment of Animals. Mastery of Inoculation Technique

Assignment 1. To become acquainted with the organization of mass treatment of agricultural animals. 2. To master the technique of injecting biologicals by different methods. 3. To make up lists of animals and documents for the inoculations.

Basic Equipment and Materials. A set of syringes (one, two, five, 10, 20, 150, 200 cc) and needles for them, sterilizers, cotton, gauze, forceps, a Primus or electric stove, an Agali [?] cock, equipment for intravenous injection, sterile physiological saline, scissors, instruments for holding the animals, three percent phenol solution. Documents and lists.

Animals: horses, cattle, hogs, sheep, goats, dogs, chickens, turkeys, ducks and geese.

Place of the Exercise. The exercise is best held at a meat combine (on cattle ready for slaughter) or in an animal husbandry farm: in an extreme case, in the clinic of the technical school.

Method of Conducting the Exercise. The instructor acquaints the students with the organization of mass treatment of agricultural animals (inoculations, allergic examinations, taking of blood and others), directs attention to the need for making a clinical examination of the animals before treatments, proper equipping of the place for inoculations and placement of the persons involved.

After this, the instructor mentions to the students the methods of fixation of the animals, shows how to prepare the injection area and demonstrates various methods of injecting biologicals.

The students are divided into groups of four. In each group the duties are divided as follows: two hold the animals down, one prepares the injection area, and another injects the preparation. During the course of the exercises the students change places. Each student should master the technique of injecting biologicals by different methods. In place of the biologicals students are given sterile physiological saline. The instruments are prepared by the students themselves. The instructor checks on the correctness of the work.

In conclusion, the instructor tells how to make up the documentation for the treatment. The students make up the list of animals by themselves, make out the documents for inoculations, and give

them to the instructor for checking.

Note. The assignment for making up documents can be given to the students at home with subsequent checking of the manner of performance of the assignment by the instructor.

Organization of Mass Treatment of Agricultural Animals. Mass treatments of agricultural animals (inoculations, investigations, taking of blood and others) are conducted with the aim of prophylaxis, diagnosis or elimination of infectious diseases.

The prophylactic measures are taken on the basis of a plan of veterinary-sanitary measures which is worked out by the chief veterinary physician of the region (or farm) after careful study of the epizootic state of the region (or farm).

In organization of inoculations the possibility of giving active or passive inoculations in a given farm should always be determined.

Before beginning the inoculations a clinical examination of the entire herd is conducted; in the case of compulsory inoculations thermometry of the animals is obligatory. Animals with an elevated temperature are not vaccinated; they are given immune serum, and after a certain time they are inoculated with vaccine.

Some vaccines (live vaccines against anthrax and others) should not be given to weak, debilitated animals or to those in the last months of gravidity or shortly after delivery.

It is necessary strictly to observe the established intervals between injections. In a fixed focus of infection it is necessary to check on timely follow-up inoculations of the growing young (for example, in calf paratyphoid) and inoculation of the herd which has newly come into the farm.

Setting Up the Place for Treatment of Animals. For the purpose of conducting mass treatments special premises, sheds and corrals are set up. On this area an instrument table is set up. Large animals are tied to picket lines or placed in a stall. It is convenient to use a branding-chute (Fig 56), at which posts are set up at the place of treating the animals for the purpose of tying them; two transverse poles are set between the posts which limit the movement of the animal forward and backward (in front, at the level of the carpal joint and middle third of the neck; behind, at the level of the hock and thigh).

For mass inoculations hogs are driven into close quarters in batches of 30-40 head.

The success of the inoculations depends on the proper arrangement of the people. One of the assistants prepares and disinfects the injection site; another disinfects this place after injection of the

Fig 56. Branding-Chute.

preparation and marks the inoculated animal. An assistant is also needed who will keep a record of the inoculated animals.

It is compulsory to mark inoculated animals; for this, tinctorial solutions can be used. It is convenient to use the following technique: the injection area prior to the injection is treated with three percent phenol solution, and after the injection with tincture of iodine, which is readily seen on the hairy integument of the animals (particularly in hogs) and remains for several days.

Fixation of the Animals. The methods of fixation of the animals depend on the species of animal and the nature of the treatment.

In the case of inoculations of horses in a number of cases the fixation is limited only to the head. In the case of refractory animals one of the anterior extremities can be raised and a clamp can also be applied to the upper lip or ear. Sometimes, recourse is had to fixation in special stalls or branding-chutes.

Fixation of cattle consists of holding the animal by the horns or by the nasal septum (nasal forceps can be used).

Young pigs are held by raising up their hind extremities. In the case of mass treatments it is sufficient to drive the hogs into close quarters. Sometimes, the hog is fixed by the application of a loop of rope to the upper jaw. For this purpose metal forceps can be used by means of which the upper jaw is compressed.

Sheep are held in branding-chutes or rolled over by their extremities.

In treating dogs a gauze ligature is applied to both jaws the ends of which are tied on the nape of the neck.

A bird is held by the wings and extremities. The bird can be held at the axilla, and its head can be held with the other hand.

Methods of Injecting Biologicals. The biologicals are carefully shaken up before using; the serum is heated in the water bath to a temperature of 36-38° before intravenous injection; with subcutaneous injection it is heated to room temperature.

Biologicals are taken up in a syringe from the bottle. For this purpose the sealing wax and the stopper are removed, the upper part of the bottle is wiped with disinfectant solution, and the stopper is pierced with a sterile needle and the preparation is taken up into the syringe through it (the needle is left in the stopper until the preparation in the bottle is completely used up). In using a system with the Agali cock the stopper with the rubber hose is transferred from one bottle to the next. It is forbidden to pour biologicals into another container.

Each animal is inoculated with a separate sterile needle. In

the case of horses, cattle and deer the biologicals are injected in the area of the middle third of the neck; in camels, in the area of the lower third of the neck; in hogs, sheep, goats and dogs, in the area of the inner surface of the thigh or inner surface of the foreleg; in birds, into the pectoral muscle. Preparations can be injected into hogs in the region of the neck at the base of the ear.

Allergic preparations are applied to the conjunctiva or injected intradermally (less often, subcutaneously). Intradermal injections of allergens are given to calves in the middle third of the neck; to calves and sheep, into the subcaudal fold; into hogs and sheep, in the skin of the ear; chickens, in the skin of one of the barbs. At the injection site there should be no abrasions, ulcers, abscesses or others. The hair at the injection site is shaved off; the skin is treated with three percent phenol solution. The injection site is smeared with tincture of iodine after the puncture.

Large doses of serum are injected in several places with subsequent light massage.

1. Subcutaneous method. The syringe is taken in the right hand; the skin fold is taken up in the left hand, pulled out slightly, and the needle is injected into the triangle formed. With correct injection the needle tip can be freely rotated under the skin.

2. Intramuscular method. The syringe and needle are directed perpendicular to the surface of the skin, and the needle is injected into the muscle layer to a depth of no less than three centimers (in swine the thickness of the fat layer needs to be considered).

3. Intravenous method. Intravenous injections in long-horned cattle and horses are given in the jugular vein in the middle third of the neck. It is more convenient to give the injection on the left side.

The container (the barrel of a Janet syringe or glass funnel) with the fluid introduced into it is covered with a sterile gauze napkin. The assistant holds the container in such a way that the end of the hose is above the fluid level in the container.

After treating the injection site, the jugular vein below this place is compressed with the left hand and, after waiting until the vein stands out prominently, the needle is injected into the vein in the direction toward the head. The assistant holds a tray under the needle so that the blood does not fall to the ground.

After blood comes out of the needle, the left hand is dropped and the tip of the rubber hose from the container with the biological is attached to the needle. For this purpose the assistant gives the tip of the hose to the operator and he himself lets the container down

by the same token removing the air from the hose and filling it with the preparation. The hose is attached to the needle, and the assistant slowly lifts up the container, thereby observing the entrance of the fluid into the vein through the glass cannula in the hose.

After noting the passage of the last portion of the preparation through the glass tube the rubber hose is disconnected and the needle is washed with blood. The injection site is disinfected with tincture of iodine.

4. Intradermal method. For intradermal injections fine needles are used (Nos 28-32). At the prepared site the skin is taken up with two fingers of the left hand and the needle is inserted into the fold. The needle should be directed from above down at an acute angle to the skin surface. The appearance of a small swelling (the size of a pea) which is absorbed after several minutes serves as a check on the correctness of the intradermal injection at the injection site.

4. Percutaneous method. This is used for vaccination of poultry against fowl pox. The diluted vaccine is rubbed into the feather follicles (before this the feathers are plucked out).

6. Application of the biologicals to the conjunctiva is carried out by means of eyedroppers. The eye is held open with the left hand, and two-three drops of the preparation are introduced into the pocket formed by the lower lid.

7. Oral administration of the biologicals is conducted by means of rubber or glass bottles or syringes with a long rubber tube as well as by means of adding the biological to the drinking water.

The inoculated animals are observed, and sometimes their temperatures are taken. After the inoculations, particularly with living vaccines, it is essential to see that the animals are not subjected to chilling, overheating, that they do not go on long drives and are not kept in a crowded manner. During the inoculations or diagnostic examinations the movement of animals from one group into another is forbidden.

When complications from the inoculations are demonstrated it is necessary immediately to stop further use of the preparations, to give aid to the sick animals (injection of specific serum), and let the chief veterinary physician of the region know about it. A document is made out about what has happened which is sent, along with two unopened bottles of the biological, to the State Scientific Control Institute of Veterinary Biologicals.

A list is made up of all the animals treated with an indication

of the time and nature of treatment, the name and series of biological, the biological plant which prepared the biological, the state inspection numbers, the names of the inhabited place, the numbers, "names", distinctive markings, sex and age of the animals, doses and methods of administration of the preparation. In this list the complications determined in the animals during the observation period are noted.

Exemplary Form

List of animals inoculated against black quarter. Date of inoculations, 25 March 1960. Characteristics of the biological used: formol vaccine for black quarter, series 26, prepared by the Omsk Biological Plant, state inspection No 101.

[The Table reads as follows: the title is Inhabited Place (or Farm) -- Dyad'kovo Village in Glukhovskiy Rayon. The headings, reading across, are as follows: No, No or "name" of animal; distinctive markings, color; sex; age; dose of preparation; mode of administration; complication. Reading across opposite 1: No 164; black-mottled; female calf; one year; five cc; subcutaneously.]

After a certain time (usually 15-30 days) the results of the treatments are read, and a document is made up. In this document the name of the inhabited place and farm, the date of the inoculation, who gave the inoculation and what animals were given it, their number according to species and ages, the biological used for the inoculation (name of the biological, series, biological plant which prepared the preparation, longevity, state inspection number), how the preparation was injected, how the injection site was treated and how the instrument was prepared, doses of the biological, total expenditure of biological, existence of complications and their nature are indicated. The document is signed by the veterinary and zootechnical specialists as well as by the manager of the farm or brigade leader.

Exemplary Form

Document

28 October 1960

[The document is a set form which answers the specific questions

given in the last paragraph. It reads as follows]

Proletariy Sovkhoz, Belyana Village, Farm No 1.

We, the undersigned veterinary technician of the farm A. I. Fedorov and zootechnician G. P. Kuz'min, and brigade leader I. I. Klimov, have made up the present document to the effect that during the period from 10 to 14 October 1960 the long-horned cattle of the farm were inoculated against anthrax. A total of 250 cows was inoculated. The inoculation was given with STI anthrax vaccine, series 50, prepared by the Kaluga Biological Plant, suitable for use until January 1961, state inspection No 50. The preparation was given subcutaneously. The injection site was treated with three percent phenol solution. The instruments were sterilized by boiling for 20 minutes. The preparation doses for the cows were 1.5 cc each. The total consumption of biological was 375 cc. No complications were observed as the result of using the biological. A list of the inoculated animals is appended.

Signed: A. I. Fedorov, G. P. Kuz'min and I. I. Klimov.

Exercise 16

The Use of Biologicals and Antibiotics for the Treatment of Animals with Infectious Diseases

Assignment. 1. To master the technique of injecting sera for therapeutic purposes. 2. To inject bacteriophage into the animal (calf or young pig); 3. To dissolve the antibiotics and inject them into the animal.

Basic Equipment and Materials. Syringes of 10, 20 and 200-cc capacity, needles, a system for intravenous injection of the sera, rubber hose, sterilizer. Sterile physiological saline solution, immune sera, bacteriophages, antibiotics, three percent sodium bicarbonate solution in boiled water, three percent phenol solution. Scissors, cotton. Water baths.

Animals: Horses, calves, young pigs and dogs.

Place of the Exercise. The clinic or vivarium of the technical school. During the class practice the exercise may be conducted in an animal husbandry farm.

Method of Performing the Exercise. The instructor briefly acquaints the students with the use of immune sera, bacteriophage and antibiotics for the treatment of animals with contagious diseases.

The students are divided into groups of three: independently, they inject the animals with sera by different methods (subcutaneously, intramuscularly, intravenously), dissolve the bacteriophage after preliminary administration of sodium bicarbonate solution and inject it into the calf or young pig, dissolve and inject the animals with various antibiotics. Instead of sera the students may be given sterile physiological saline.

Treatment of Animals with Contagious Diseases

In a number of infectious diseases the animals are given medical assistance. However, there are contagious diseases in which the treatment of the animal is inexpedient or is connected with the danger of spread of the infectious disease as well as infection of man (glanders, tuberculosis, brucellosis and others). In such cases no treatment is given to the animals; actions are taken with them according to instructions for control of certain diseases.

The agents used for the treatment of animals with contagious

diseases are divided into specific and symptomatic. Among the specific agents are immune sera, antibiotics, sulfonamides and chemotherapeutic preparations. In giving therapeutic aid it is necessary to keep in mind the danger of the animals with infectious diseases for the rest of the herd and in a number of cases for man also.

The Use of Immune Sera for the Treatment of Animals with Infectious Diseases. Immune sera in certain infectious diseases are used for prophylactic and therapeutic purposes. The therapeutic dose of serum, as a rule, exceeds the prophylactic dose by two times. Before using, the serum is shaken up and heated in a water bath to 38°. The best therapeutic effect is given by the serum in the initial period of the disease. There are several methods of using the sera: 1. Subcutaneously; 2. Intramuscularly; 3. Intravenously.

The technique of injecting sera by these methods has been described in Exercise 14.

For better absorption of the serum, no more than 50 cc of the preparation should be injected in the same spot subcutaneously or intramuscularly; after injection of the serum the injection site should be rubbed slightly (massaged).

In the case of a disease with a severe course the serum is injected repeatedly depending on the general condition of the animal.

In order to avoid anaphylactic shock it is recommended that the desensitizing dose of the preparation be given before injection of the full serum dose (0. 3-0. 5 cc in small animals; one-two cc in large animals), and that the full dose of serum be injected after 15-20 minutes.

The Use of Bacteriophage. Bacteriophage is used for prophylactic and therapeutic purposes for paratyphoid in calves and young pigs, colibacillosis and mixed coli-paratyphoid infection. There are no contraindications to the use of bacteriophage.

The use of bacteriophage does not exclude symptomatic therapy, serotherapy (the use of specific sera) or the taking of measures for the improvement of the zoological-hygienic conditions of maintenance and feeding of the young animals as well as measures for the control of paratyphoid and colibacillosis in the young in accordance with existing instructions.

Phage therapy of the animals sick with paratyphoid or colibacillosis exerts the maximum effect during the initial period of the disease. Before using the bacteriophage the animals are given a starvation diet for four-eight hours; thereby, only water for drinking is given. Ten to 15 minutes before administration of the phage the

sick animal is given 25-30 cc of three-five percent sodium bicarbonate solution in boiled water by mouth.

Directly before administration bacteriophage is diluted in 100 cc of boiled water which has been allowed to cool to a temperature of 20°. The bacteriophage is administered orally in a dose of 30-50 cc three times every two hours. In severe cases of the disease the quantity of bacteriophage given can be increased to 100 cc per dose.

Bacteriophage may be administered from a bottle or from a syringe with a rubber hose.

In the case of phage treatment attention is paid to the diet. Acid foods should be excluded from the diet during the treatment period. After three days of treatment with the bacteriophage a one-two-day interruption is made, and when necessary the treatment is repeated.

Subcutaneous or intramuscular injection of bacteriophage in a dose of 30-50 cc once a day is possible. When necessary, the injections are repeated in the same doses no sooner than after 24 hours. In giving bacteriophage by mouth laxatives or disinfectants should not be used simultaneously.

The Use of Antibiotics. At the present time, in certain infectious diseases various antibiotics are used successfully for therapeutic purposes. The effectiveness of use of antibiotics depends on the correctness of choice of the preparation, the mode of administration and the dose. Antibiotics exert the best therapeutic effect in the initial period of the disease.

In veterinary practice penicillin, ecmonovocillin, synthomycin and levomycetin, biomycin and terramycin are widely used [ecmonovocillin is ecmoline, an antibiotic derived from fish, plus novocaine-penicillin; levomycetin, levo-rotary chloromycetin; synthomycin is racemic chloromycetin; biomycin is aureomycin].

With the use of antibiotics for the control of contagious disease in agricultural animals, including poultry, all measures are taken provided by the instructions. Symptomatic treatment should be conducted independently of the use of antibiotics.

For the treatment of animals with contagious diseases antibiotics are used orally, subcutaneously, intramuscularly and intravenously. The technique of injecting the antibiotics is similar to that described in Exercise 14 and in the present Exercise (see bacteriophage).

The doses of antibiotics are calculated per kilogram of the animal's weight in units (or milligrams).

Penicillin. It is used for swine erysipelas, strangles in

horses, necrobacillosis, anthrax, and malignant edema [bradzot]. Penicillin is put out in bottles on whose labels the number of units and the longevity of the preparation are indicated.

Penicillin is dissolved in sterile physiological saline, distilled or boiled water (which has first been cooled) directly in the bottle. For this purpose the necessary quantity of the solvent is taken up with a sterile syringe. The rubber stopper of the bottle is wiped with cotton moistened in alcohol, and the bottle stopper is pierced with the needle. The needle should enter the bottle a distance of one-two millimeters beyond the stopper. After this, the solvent from the syringe is introduced into the bottle, shaken up and after complete solution of the preparation the necessary quantity of the penicillin solution is again taken up into the syringe. Thereby, the syringe is held vertically; the bottle should be above the syringe. After taking up the necessary amount of solution, the needle is withdrawn from the bottle stopper. Penicillin in solution can be kept for no more than 24 hours.

Penicillin solution should not be sterilized, because this leads to destruction of the preparation. Penicillin should not be mixed with acids or alkalis, alcohol, potassium permanganate, hydrogen peroxide, salts of heavy metals or iodine solutions.

Penicillin should be injected subcutaneously or intramuscularly; the use of penicillin for irrigation of cavities is possible (for example, for irrigation of an abscess cavity in the case of strangles in horses).

In the treatment of contagious diseases in animals penicillin is used in accordance with the indications, two-three times a day with intervals of three-six-12 hours. The use of penicillin is stopped three-four days after clinical recovery of the animal. If, during the first two-three days of penicillin treatment, no noticeable improvement occurs in the condition of the animals further use of penicillin is stopped, or it is given in combination with other antibiotics.

Ecmonovocillin. The preparation is a suspension of the novocaine salt of penicillin in an aqueous solution of the antibiotic ecmoline. A single injection of the preparation assures a therapeutic concentration of penicillin in the blood for 30-36 hours.

Ecmonovocillin is packed and distributed in units of two bottles: one containing the novocaine salt of penicillin; the other with ecmoline solution (Fig 58). Before injection, a suspension of novocaine salt of penicillin (300, 000 or 600, 000 units) is prepared in the

ecmoline solution (2.5 or five cc). For this purpose the stopper of the bottle containing ecmoline is pierced with a sterile needle on a sterile syringe (a large needle should be used). After aspirating the necessary quantity of ecmoline, the stopper of the bottle with penicillin is pierced, and ecmoline solution is introduced into the bottle. Vigorously shaking the body, a uniform suspension of a milky-white color is obtained. The suspension is rapidly sucked up into the syringe, and the animals injected intramuscularly. Intravenous injection of the preparation is not permitted.

All manipulations for the preparation and injection of the preparation into the animal are carried out quickly and with constant shaking of the syringe to avoid the formation of a precipitate and plugging of the cannula of the syringe or needle.

Indications for the use of ecmonovocillin are the same as for the use of penicillin.

Synthomycin and Levomycetin. These preparations are used for paratyphoid and colibacillosis in calves and young pigs. These preparations are given by mouth in the form of an aqueous suspension, calculating one gram of synthomycin or levomycetin per 10 cc of water (or milk); the preparations can be given in the fodder. Synthomycin is first ground up in a mortar until a fine powder is obtained.

Fig 58. Preparation of Ecmonovocillin: a. Novocain salt of penicillin and ecmoline; b. The bottle stopper is rubbed with alcohol; c. Ecmoline is sucked up into the syringe; d. Ecmoline is injected into the bottle containing penicillin; e. The emulsion ready for use is sucked up into the syringe.

Before giving the suspension to the animal it is carefully shaken up. The suspension is injected by means of a syringe furnished with a rubber hose.

Synthomycin or levomycetin or used in a dose of 0.02-0.04 milligram per kilogram of the animal's weight two-three times a day with an interval of four-six hours. The preparations are continued (two-three times a day) even after clinical recovery of the animal in order to prevent possible recurrences of the disease.

Biomycin is used for paratyphoid infections and pullorosis in chicks.

The preparation is given in the form of aqueous suspensions of solutions (the solubility of the preparation in water is 10-13 milli-

grams per cc) two-three times a day calculating 20 milligrams per kilogram of the animal's weight. Biomycin is given by mouth; intramuscular use is also possible.

Terramycin is used for pasteurellosis of poultry. The preparation dissolves readily in distilled water; however, in the light the terramycin solution rapidly decomposes, in connection with which it should be used immediately after preparation.

Terramycin is injected intramuscularly for therapeutic purposes in a dose of 20 milligrams per kilogram of the bird's weight once a day three-four days straight. For use, one-two percent aqueous solutions of the preparation are made.

Exercise 17

Apparatus and Mechanized Equipment used for Disinfection

Assignment. 1. To become acquainted with the setup and use of apparatuses for disinfection. 2. To master the rules of care of disinfection apparatus. To disassemble, clean, lubricate and assemble a sprinkler. 3. Demonstration of disinfection chambers. 4. To study precautionary measures in conducting disinfection.

Basic Equipment and Materials. The disinfection apparatus: sprinklers, "Avtomaks" apparatus, "Pioner" apparatus, LSD (disinfection apparatus of the Laboratory of Sanitation and Disinfection), DUK (Komarov's disinfection apparatus).

Disinfection chambers or mock-ups (or diagrams) of them. A protective suit for conducting disinfection: a hood, overalls, gown, apron, rubber boots, rubber gloves, protective goggles or gas mask.

Place of the Exercise. A lecture-room or clinic of the technical school. The exercise can be conducted in a disinfection detachment of a veterinary-bacteriological laboratory.

Method of Conducting the Exercise. The instructor acquaints the students with the setup and the use of disinfection apparatus. The apparatus is demonstrated in action, utilizing water instead of disinfectant solution. Then, the instructor explains to the students the rules of care of the disinfection apparatus. Disinfection chambers are shown in action where possible. In the absence of chambers mock-ups or diagrams are used.

In conclusion, the instructor acquaints the students with precautionary measures for conducting disinfection, demonstrates the protective suit of the disinfection detachment worker.

Sprinklers. Sprinklers of various types are used. The sprinkler consists of a tube inside which there are suction and force pumps (valves) and a piston. A rod is attached to the piston, to which, in turn, the handle is attached. The sprinkler is operated by pressure on the handle.

In the sprinkler tube there are two holes for the attachment of suction and discharge hoses.

The suction hose serves for obtaining the disinfection solution from the container (bucket or barrel); the hose ends in an expansion filter.

The discharge hose should be adequately long and elastic.

This assures convenience in using the sprinkler. A sprayer or tip is attached to the discharge hose. Tips can provide for discharge of the solution either as a steady stream or can break it up into fine sprays (sprinkler spouts).

Most suitable for veterinary practice is the metal "Kostyl'" fire-fighting type sprinkler (Fig 59). It is distinguished by great force; it ejects a stream for 10-15 meters. After work, the sprinkler should be carefully washed with water, disassembled, wiped dry and lubricated.

Fig 59. The "Kostyl'" Sprinkler: 1. Pump handle; 2. Connecting piece for attachment of suction hose; 3. Connecting piece for attachment of force hose; 4. Cart.

"Avtomaks" apparatus or the garden sprinkler can be used in veterinary practice for disinfection of animal husbandry premises. The apparatus consists of a metal container in the cover of which a pump is set for pumping air into the container. There is an opening in the wall of the apparatus to which a rubber hose is attached, and a sprayer is attached to that. The apparatus is carried on the back by straps.

The "Pioner" apparatus is designed for control of agricultural pests, but can be used for the disinfection of animal husbandry premises. The apparatus has suction and force pumps and a tank of 440-liter capacity for the disinfectant solution. Two discharge hoses yield 18.6 liters of disinfectant solution per minute. The "Pioner" apparatus is carried on a truck or on horses.

The LSD disinfection apparatus is designed for disinfection of animal husbandry premises with cold and hot solutions, emulsions and suspensions. The apparatus has a four-cycle engine, a pump which creates a pressure of four-five atmospheres, a reservoir (boiler) for disinfectant solution, a discharge hose with sprayers and fuel for heating the disinfectant solution.

The LSD apparatus is mounted on a metal frame which makes it possible to carry it on a truck or on sleds (Fig 60). In working with the LSD disinfection apparatus there is a certain sequence used.

1. The motor of the apparatus should be set at 1450 revolutions a minute. 2. The reservoir of the boiler should be filled, and the solution of disinfectant prepared (Fig 61). a) The suction hose is connected into a connecting piece (10) and the handle is set on the position "suction from body of water"; b) The water is poured

in through the vertical nozzle (11) of a T-piece (or valve); c) The handle of the valve (8) should be put in the "reservoir-filling" position; d) The motor should be turned on and the filling of the reservoir should be watched by the water gauge; e) Without turning off the motor, the handle of the valve (10) should be set in the "suction-from-reservoir" position, and the necessary quantity of disinfectant should be poured through a funnel into the opening in the boiler (1). 3. By means of a valve (2) the pressure is set at four-five atmospheres. 4. The force hose is connected to the connecting piece (6); the valve (8) is shut off and the disinfection is begun.

Fig 60. LSD Disinfection Apparatus Mounted on Auto Trailer (General Appearance).

Fig 61. Diagram of LSD Disinfection Apparatus: 1. Hatch for pouring in disinfectants; 2. Safety valve; 3. Thermometer; 4. Connecting piece for connection of discharge hose; 5. Manometer; 6. Connecting piece for attachment of discharge hose; 7. Opening for cleaning boiler (reservoir); 8. Three-way stopcock (pressure main); 9. Three-way stopcock (suction piping); 10. Connecting piece for connection of suction hose; 11. Vertical nozzle. [The words on the figure reading from above left to lower right are: fuel, boiler, pump, motor].

In working with hot solutions the force hose is connected to the connecting piece of a coil (4). The handle of the cock (8) is placed in the "fluid-heating" position and the motor is turned on. The smokepipe is set up and the fuel is burned. When the fluid reaches the necessary temperature disinfection is begun.

N. M. Komarov's Automotive Disinfection Apparatus (DUK) is designed for disinfection and insect elimination in animal husbandry premises. Disinfection by means of this apparatus can be conducted with cold or hot solutions of disinfectants. The latest model of the apparatus is equipped with an apparatus for aerosol disinfection. The apparatus can be set up on the chassis of a GAZ-51 or GAZ-63 truck (Fig 62).

Fig 62. Komarov Disinfection Apparatus (DUK) (General Appearance): 1. Tank for water or solutions; 2. Tank for initial disinfectant solution; 3. Boiler for heating the water and disinfectant solution; 4. Accessory cab for service personnel; 5. Boxes for hoses, instru-

ments and material; 6. Manometer for measuring pressure in the tank.

The main parts of the apparatus are: 1. A tank for water or solution with a capacity of 1.2 cubic meters (1); 2. Suction-distributing piping with three valves (for the suction hose, for the discharge hose, and for the purpose of connecting the tank with the boiler); 3. Two tanks for the basic disinfectant (2); 4. A boiler for heating the water and disinfectant solution connected with the tank; it is equipped with fuel and a throw-over smokepipe (3); 5. A suction hose for filling the tanks with fluid (or water) with a filter screen and a grid at the tip; 6. Discharge hose with nozzles; 7. Accessory cab for service personnel (4); 8. Boxes for hoses, material, equipment and instruments (5); 9. Manometer for measuring pressure in the tank (6) and a thermometer for measuring the temperature to which the fluid is heated in the boiler.

The apparatus has units which create negative and positive pressures in the tank (vacuum piping and pressure piping).

Setting Up the DUK and Working with It. Before going out the tanks are filled with the basic disinfectant solution; a supply of firewood is put into the boxes.

The disinfectant solution is prepared in the main tank [1 on on Fig 62] by means of aspirating the basic disinfectant solution into it from the smaller tank [2 on Fig 62] and water from a water body. For this the following are necessary:

1) to start the motor; 2) to connect the suction hose (13) to the valve (5) (Fig 63) of the suction piping and to drop the ends of the hose into the body of water; 3) to open the valve (3) of the vacuum piping; 4) to open valve (5); 5) to open the valve connecting the main tank with the smaller tank of basic disinfectant solution (10).

As soon as the main tank is filled with the necessary quantity of water (the tank filling is watched by the water gauge(21)) the following must be done:

1) the valve (3) of the vacuum piping must be closed; 2) the valve (5) of the suction piping and the valve of the smaller tank need to be shut off; 3) the suction hose (13) should be taken out of the water body and disconnected; 4) the negative pressure should be eliminated in the tank by means of opening the valve (2) or (8).

Fig 63. Diagram of DUK Apparatus: 1. Muffler cut-out valve; 2. Pressure piping valve; 3. Vacuum piping valve; 4. Valve for heat conduit of tank; 5. Valve of suction piping; 6. Valve of distribution

piping; 7. Valve of distribution piping and of connection with boiler; 8. Valve for connection with water supply; 9. Valve for connecting the boiler with the discharge hose; 10. Small tank for basic disinfectant solution; 11. Boiler for heating disinfectant solution; 12. Discharge hose with nozzle; 13. Suction hose with nozzle; 14. Muffler; 15. Automobile motor exhaust pipe; 16. Small tank for basic disinfectant solution and valve for connection with aerosol sprayer; 17. Main tank for disinfectant solution (or water); 18. Manometer; 19. Safety valve; 20. Thermometer; 21. Water gauge.

If the main tank is filled with water from a water supply line the suction hose is connected to valve (8) and the other end of the hose to the water supply line. In order to keep the pressure from rising in the main tank thereby, it is necessary to open valve (2). After filling the main tank the valves(2) and (8) should be shut off and the suction hose taken up.

After this, the apparatus is prepared for operation (it is set up near the object to be disinfected, the discharge hose is connected with the sprayers). In the main tank a positive pressure of two-2.5 atmospheres is created, which is achieved by compression of exhaust gases of an automobile motor. For the purpose of establishing the necessary pressure it is necessary: 1) to start up the motor; 2) open the valve (2) of the pressure piping; 3) shut off the muffler cut-out valve (1); 4) as soon as the manometer shows a pressure of two-2.5 atmospheres the valve (2) should be shut off, the motor should be shut off, and valve (1) should be opened. The apparatus is ready for operation. The valve (6) is opened, the disinfectant solution is fed to the discharge hose, after which the disinfection is begun.

In working with hot solutions, the boiler (11) serving for heating the fluid is filled with disinfectant solution after the creation of pressure in the main tank. Then, the furnace is ignited, and the solution in the boiler is heated to the necessary temperature. The discharge hose in this case is connected to the valve (9) and disinfection of the object is begun.

During work the temperature of the solution and the pressure are watched. With reduction in the pressure the motor is turned on periodically; for the purpose of increasing the temperature the fire is increased in the furnace.

Operation of the DUK is stopped in the following sequence: 1) the fire in the furnace is put out; 2) the remaining disinfectant solution in all containers is poured out; 3) the main tank, boiler and

hoses for pure water are carefully washed out (by sucking up water and emptying the tanks and hoses two times); 4) for definitive removal of the water from the apparatus pressure is created in the main tank, and all the drain cocks are opened. During the cold season it is also essential to let the disinfectant solutions out of the small tanks.

Disinfection Chambers. Three types of chambers are distinguished: hot air, steam and gas.

An example of the hot air chamber is the dryer (Pasteur oven) whose description is given in Exercise 6. In practice the Levinson-Chernoshchekov apparatus is used which consists of the chamber and an oven forming the base of this chamber. Because of a special arrangement hot air circulates in the chamber.

The simplest chamber for steam disinfection is the barrel-chamber. It consists of an ordinary boiler into which water is poured. Below the boiler a furnace is placed. The chamber proper is a wooden barrel with holes pierced in the bottom, which is set up on the boiler. In the chamber (barrel) objects are placed which are to be disinfected. Through the holes in the bottom of the barrel live steam penetrates into the chamber and disinfects the objects.

An example of a gas chamber is the steam-formalin chamber, in which objects are exposed to the simultaneous effect of steam and formalin. Steam-formalin disinfection can be used for the treatment of animal husbandry premises. Disinfection is carried out with a Flügge apparatus, which is a container into which formalin diluted with water is poured. The container is set up on a special support and heated from below with an alcohol burner. Through a hole in the top of the container the vapors of the boiling formalin enter the room.

Precautionary Measures for Disinfection. In conducting disinfection it is necessary to protect people carrying out the disinfection as well as animals against the harmful effects of the chemical agents. Persons who are carrying out the disinfection should be provided with thick special suits (hoods, overalls, rubber gloves, rubber boots and gowns). In disinfection with chlorine and formalin preparations the work is done in gas masks. In working with solutions of caustic alkalis and acids protective goggles should be used; in order to avoid burns contact of these solutions with the skin and clothing should be avoided.

In the case of disinfection of rooms with chemical agents (caustic alkali, sulfuric-carbolic acid mixture, preparations of chlorine, formalin solutions and others) the animals must be taken outside.

Two or three hours after disinfection the feeding troughs and

partitions in the stalls should be washed with water. The room in which the disinfection was carried out should be aired out well and the animals can be brought in only after this.

Exercise 21

Anthrax. Diagnosis of the Disease

Assignment. 1. To take pathological material from a dead animal for investigation for anthrax. To pack it for shipping to a laboratory and write out the accompanying document.

2. To prepare smear-impressions from the organs of a dead mouse, which died as the result of infection with Tsenkovskiy's second vaccine; to stain the smears by the Gram method and for capsules.

3. To examine the prepared stain-smears of anthrax cultures under the microscope. To draw a picture of what is observed under the microscope.

Basic Equipment and Materials. Dead body of an animal which died of a noncontagious disease. Dead bodies of mice which died as the result of infection with Tsenkovskiy's second vaccine (the instructor infects the mice two-three days before the exercise, using 0.2-0.3 cc of the vaccine subcutaneously). Scalpels, scissors, spatulas for cauterization, forceps, trays, sterilizers, a Primus or electric stove, strong binder twine, glass slides, matches, parchment paper, gauze or cloth, oilskin, glass vessels with covers (better ground), a metal or wooden box with sawdust, sealing wax or paraffin, three percent phenol solution. Sets of stains for Gram staining and for capsule staining (after Holt, Mikhin and others). Ready-made stain-smears of anthrax cultures. Microscopes, cedar oil. Gowns, rubber gloves.

Place of the Exercise. Laboratory and clinic of the technical school.

Method of Performing the Exercise. The instructor briefly mentions to the students the signs (clinical and pathological) suspicious of anthrax, explains the method of taking pathological material for examination for anthrax, directs attention to the observance of precautionary measures for preventing the spread of infection and infection of man.

The students are divided into groups (the number of groups depends on the number of dead animal bodies; if there is one body, the work may be done in two groups). Each student prepares no less than two thick smears of blood from the peripheral vessels and packs them for shipping to the laboratory. Students of each group take an

ear from the animal and also pack it for shipping to the laboratory. If a dead pig is available the retropharyngeal lymph nodes should be used for the investigation.

Then the students prepare smear-impressions from the organs of dead mice, which died as the result of infection with Tsenkovskiy's second vaccine, and the smears are stained by the Gram method and for capsules. After this, the students examine the prepared smears independently under the microscope as well as the prepared stain-smears of anthrax cultures, and draw the picture that they see under the microscope.

The Taking and Shipping of Pathological Material for Anthrax Examination. When anthrax is suspected the pathological material is immediately sent to the laboratory for examination. Suspicion of anthrax arises: a) in sick animals when there is marked depression, high temperature, bloody urine, a stormy course of the disease (in hogs, in the presence of a high body temperature and edema in the pharyngeal area); b) during examination of the dead body of the animal if there is no rigor mortis, in the presence of considerable swelling of the cadaver and bloody excretions from the natural orifices.

It is forbidden to dissect the bodies of animals suspected of anthrax.

For the purpose of examining the animal's body blood is taken from the vessels of the ear or other peripheral vessels; a tray or iron sheet is placed under the ear so that blood does not fall on the ground. The site of the incision is carefully disinfected, and after taking the blood it is cauterized by fire or with an incandescent metal object. The blood vessel is incised with a sterile instrument. Blood is applied to a glass slide in a thick layer (as thick a drop as possible), and then it is dried in air (not fixed!). Four smears are prepared from the blood of one dead animal, and they are set out in pairs (with the sides on which the blood has been applied facing each other), placing pieces of matches between the glass slides. The smears are carefully packed in parchment paper, tied with binder twine and sealed.

The ear of the dead animal can be sent for examination, whereby an ear is taken from the side on which the cadaver lay. A tray or iron sheet is placed under the ear. Two tight ties of binder twine are made -- one at the base of the ear; the other, one-1.5 centimeter behind the first. The ear is cut between the ties. The site of incision on the ear and on the cadaver is immediately cauterized with incandescent iron. Without removing the tie the sectioned ear is wrapped in gauze or cloth dipped in three percent phenol solution; it

is wrapped with parchment paper or oilskin and placed in a glass container (best with a ground-in stopper). The stopper is poured over with sealing wax or paraffin, and the vessel is packed in a metal or wooden box. The box is tiedwith binder twine and sealed.

Suspicion of anthrax in hogs most frequently arises during dissection when swelling and inflammatory phenomena are found in the area of the pharynx or larynx. Further dissection is stopped, and pieces of the edematous connective tissue, retropharyngeal and mesenteric lymph nodes as well as pieces of spleen are taken for examination. This material is packed in a water-proof container similar to the manner of packing the ear.

For the purpose of examination of hides for anthrax by means of the precipitin test pieces of skin measuring 10x10 centimeters are shipped.

An accompanying document is made out on the pathological material taken (see Exercise 15).

Bacterioscopic Examination for Anthrax. When the material arrives at the laboratory bactericscopy of the smears is immediately performed, on the basis of the results of which a preliminary report is issued.

Smears from the materials suspected of anthrax are stained by the Gram method and for capsules (by the Mikhin, Holt or other methods) (see Exercise 3 for the staining method).

The anthrax bacillus stains positively by the Gram method. In smears from the blood or spleen of the dead animal the anthrax pathogen is found in the form of straight rods with squared ends (Fig 66); a characteristic feature is the presence of capsules around the microbe. The bacilli are arranged singly, in pairs or short chains.

Fig 66. Anthrax Pathogen Under the Microscope.

On examination of the smears from cultures the anthrax pathogen is represented as a bacillus with slightly rounded ends. In growth on nutrient media long filaments of anthrax microbes are formed. On media to which blood serum has been added the microbes can form a capsule.

Exercise 22

Anthrax. Control Measures

Assignment. 1. To make an epizootological examination of an inhabited place (or farm) which is unfavorable with respect to anthrax and to make up an examination document. 2. To work out a plan of measures for controlling anthrax in the given inhabited place (or farm).

Guides. Instruktsiya o Meropriyatiyakh protiv Sibirskoy Yazvy [Instructions on Measures to be Taken Against Anthrax], for every student.

Place of the Exercise. Animal husbandry farm.

Method of Performing the Exercise. The instructor acquaints the students with the plan of epizootological examination of the farm and with the form of the plan of measures for the elimination of infectious disease, gives an assignment for organizing measures to be taken against anthrax.

The assignment should be based on specific circumstances (agricultural school of the technical school, the nearest animal husbandry farm).

The students may be divided into three groups and given different variants of the assignments: the occurrence of anthrax in a newly-discovered focus, at a fixed unfavorable point, the problem of eliminating anthrax in agricultural animals of different species.

Each student is given instructions for the control of anthrax. Independently the students make up the document of the epizootological examination of a farm or inhabited place, work out a plan of measures to be taken for the elimination of anthrax, make up the plan of the rayon executive committee decision for imposition of a quarantine.

At the end of the exercises the instructor makes an analysis of several of the problems solved by the students.

Through the example of anthrax the instructor should give the students a plan for the elimination of acute infectious disease in agricultural animals.

Exemplary Schema for Epizootological Examination of a Place Unfavorable with Respect to a Disease

1. Name of farm or inhabited place.
2. Number of animals (according to species).

3. Housing, maintenance, care, feeding, watering and using animals.

4. From where the cattle come for supplying the specific farm.

5. Connection with neighboring places and their status with respect to contagious diseases.

6. Whether the given farm was favorable with respect to contagious diseases in the past.

7. Whether a diagnosis was made and by what methods.

8. Characteristics of the given outbreak of infectious disease: a) when the first cases of disease appeared; b) species of animals affected; c) assumed sources of infection; d) how the disease occurred in the herd (on days and months).

9. Conclusion.

[The Table on page 145 is entitled: Plan of Measures for Elimination of Infectious Diseases; its headings read as follows: No, name of measure; time at which accomplished; one responsible for its accomplishment; who checked on it; note.]

Examples of Problems which should be Represented in the Plan of Measures to be Taken for Elimination of an Infectious Disease

1. Measures for the detection and elimination of the source of infectious disease as well as means of its influx into the farm.

2. Organization of quarantine and restricting measures provided for by instructions for this disease.

3. Which examinations of the animals should be conducted and when.

4. Isolation of sick animals and those suspected of the disease. Treatment of sick animals.

5. Giving of inoculations.

6. Time of mechanical cleaning and disinfection.

7. Security measures before lifting quarantine or removing restrictions.

Example of a Variant of the Problem in Exercise 22

Strokino Village was considered healthy with respect to anthrax, although in this region cases of this disease had been recorded among agricultural animals. On 1 August 1960 suddenly a six-year-old

cow died which belonged to the Vpered Kolkhoz, located in this inhabited place. The animal's body was kept until the veterinary specialist arrived. In the last two years the kolkhoz herd had not been supplemented by animals from other farms. There was no information on the animals of the individual herd. The animals were watered at a pond, at which the cattle of the neighboring village, Ryazanovka, also took water.

The animals had not been vaccinated against anthrax in the village of Strokino. The pathological material (blood smears) was found positive for anthrax by the laboratory to which it had been sent. On 3 August 1960, on examination of cows (80 head), which were in direct contact with the cow which died, two head were detected suspicious of anthrax.

The total cattle in the kolkhoz were as follows: 220 adults; 360 head of young; adult horses, 14; young horses, 19 head.

The cattle for personal use were distributed as follows: adult cattle, 21 head; young, 24 head.

Assignment. 1. To make up the document of the epizootological examination of the farm. 2. To work out a plan of measures to be taken for the elimination of the disease. 3. To write up the plan of the rayon executive committee decision for imposition of a quarantine.

Solution of the Problem

Document of Epizootological Examination

5 August 1960 — Strokino Village
Vpered Kolkhoz

We, the undersigned, V. V. Sidorov, veterinary technician of the Vpered Kolkhoz, I. I. Glukhov, veterinary assistant of the Kolkhoz and V. M. Kozlov, brigade leader of the animal husbandry farm have made out this document to the effect that on this date an epizootological examination has been made of Strokino Village, as the result of which the following was determined.

1. Total animals in Strokino Village.

Kolkhoz sector:

Adult cattle	220 head
Young cattle	360 head
Adult horses	14 head
Young horses	19 head

Individual sector:

Adult cattle . . . . . 21 head

Young cattle . . . . . 24 head

2. The adult kolkhoz cattle was housed in three standard cow-houses; the calves, in two standard and one adapted calf-houses. Feeding, care and maintenance were satisfactory. Watering was from the same pond.

3. The kolkhoz herd had not been supplemented from without in the past two years; there was no information on animals of the individual sector.

4. There was no connection with neighboring inhabited places. In this region cases of anthrax were recorded among agricultural animals.

5. In the past, the farm and the given inhabited place had been healthy with respect to anthrax.

6. The diagnosis of anthrax was made by laboratory studies of pathological material (blood smears from the dead animal).

7. A case of the disease was registered 1 August 1960. A six-year-old cow became sick in the first herd and died on the first day. The source of infection was not detected. The body was kept until the veterinary specialists arrived.

On 3 August 1960 two other head of animals suspected of anthrax were detected.

Conclusion. In the kolkhoz herd of Strokino Village a case of anthrax was established in cattle. Measures for the control of this infectious disease should be taken on the basis of existing instructions. The development of the plan for eliminating the infectious disease should be entrusted to the veterinary technician of the kolkhoz, Comrade V. V. Sidorov.

Signed ... Sidorov, Glukhov and Kozlov

[The Table on pages 148, 149 and 150 is entitled: Plan of Measures for Elimination of Anthrax in Strokino Village (Vpered Kolkhoz in No ___ Rayon); the headings of the Table are as follows: No, name of measures; time at which taken; person responsible; who checked on it; note. Translation of the material in the columns will be given in accordance with the number of the horizontal column as shown in the first column on the left, and the entries will be separated by a semicolon. 1. Detecting the sources from which anthrax entered the farm and taking measures to eliminate them; 5 August 1960; veterinary technician Sidorov; chief veterinary physician of the rayon.]

2. Burning the animal's body and disinfecting the place where the body was found; 5 August 1960; veterinary assistant Glukhov; veterinary technician Sidorov. 3. Writing up a plan for the rayon executive committee decision concerning imposition of quarantine and reporting to the chief veterinary physician of the rayon; 6 August 1960; veterinary technician Sidorov; chief veterinary physician of the rayon. 4. Examining and taking the temperatures of the entire herd of animals in the village of Strokino; isolating the sick animals and those suspected of disease; 6 August 1960 and every day; veterinary technician and veterinary assistant; chief veterinary physician of the rayon. 5. Mechanically cleaning and disinfecting the premises and adjacent territory; 7 August 1960 and after every excretion of the sick animals; veterinary assistant and brigade leader of the animal husbandry farm; veterinary technician. 6. Organizing guard posts; 7 August 1960; the chairman of the kolkhoz; chief veterinary physician of the rayon. 7. Organizing the individual watering of the animals; 7 August 1960; brigade leader; veterinary technician. 8. Giving serum inoculations to cows which were in direct contact with the dead animal and those suspected of the disease, vaccinating the other animals; 8 August 1960; veterinary technician; chief veterinary physician of the rayon. 9. Giving therapeutic aid to the sick animals; as they were isolated; veterinary technician; chief veterinary physician of the rayon. 10. Taking security measures: cleaning and disinfecting the premises and adjacent territory; 15 days after the last case of death or recovery of the animal; veterinary technician, brigade leader; chief veterinary physician of the rayon. 11. Lifting the quarantine and making up the document; after security measures were taken; veterinary technician, chairman of the kolkhoz; chief veterinary physician and chairman of the local council. At the bottom of the Table the following is written: plan made up by veterinary technician

(Sidorov).

Executive Committee of the Rayon Council of Workers' Deputies — Draft

Decision

No _____ — 6 August 1960

"Measures for the Elimination of Anthrax in the Vpered Kolkhoz (Strokino Village)"

After hearing the report of the veterinary technician of the Vpered Kolkhoz, Comrade V. V. Sidorov, concerning the occurrence of anthrax in the kolkhoz herd of Strokino Village, the Executive Committee of the Rayon Council of Workers' Deputies decided the following for purposes of preventing the spread of infection, being guided by the Veterinary Code of the USSR and Instructions of the Ministry of Agriculture USSR, "Measures to be Taken Against Anthrax":

1. To impose a quarantine for anthrax on the Vpered Kolkhoz (Strokino Village).

2. To declare the following inhabited places adjacent to Strokino Village endangered by the importation of infectious disease: Ryazanovka, Vasil'yevo, Polunino, Verkhniye Mayalki.

3. To oblige the chairman of the Vpered Kolkhoz, Comrade K. R. Grishin, to post two guard posts all day long on the roads leading to Strokino Village. To provide the posts with declarations of a quarantine and signs indicating detours.

4. To forbid the following during the period of quarantine in Strokino Village (Vpered Kolkhoz):

a) transportation or driving of animals through the area; driving or transporting the animals into the area; transporting or driving the animals out of the territory of the inhabited place; b) watering at the same pond (individual watering of the animals is to be organized); c) regrouping, exchange and sale of animals; d) exportation of milk or dairy products, wool, hair, hooves or other products of animal origin from Strokino Village; e) exportation of forage; f) slaughtering animals for meat; g) dissecting the dead bodies or removing the pelts from animals which die.

5. The head of the Rayon Health Department is to take measures to prevent cases of anthrax among people.

6. Anthrax should be controlled on the basis of existing instructions.

7. Responsibility for the elimination of anthrax in the Vpered Kolkhoz is to be placed on the Chairman of the Kolkhoz, Comrade K. R. Grishin, and veterinary technician, Comrade V. V. Sidorov.

8. The present decision is to be announced to all citizens who live in the inhabited places of Strokino, Ryazanovka, Vasil'yevo, Polunino and Verkhniye Mayalki.

9. Checking on the accomplishment of measures for the control of anthrax is to be made the responsibility of the deputy chairman of the rayon executive committee and the chief veterinary physician of the rayon.

Chairman of the Executive Committee
of the Rayon Council of Workers'
Deputies _____
Secretary of the Executive Committee
of the Rayon Council of Workers'
Deputies _____ /

Exercise 23

Anthrax. Vaccination

Assignment. 1. To make a clinical examination and take the temperatures of the animals. 2. To vaccinate the animals against anthrax. 3. To make up a document on the giving of inoculations and a list of the animals.

The Basic Equipment and Materials. Vaccines: first and second Tsenkovskiy's vaccines, STI vaccine, aluminum-hydroxide-precipitated GNKI vaccine against anthrax. Instructions for the use of these biologicals.

One-two-cc syringes, needles for the syringes, sterilizers, scissors, forceps, cotton, three percent phenol solution, tincture of iodine, veterinary thermometers with tail clamps, containers with disinfectant solution in which to put the thermometers, and vaseline.

Animals: cattle, horses, sheep, hogs and goats.

Place of the Exercises. Animal husbandry farm. During the practice lesson the performance of this exercise should be timed to correspond with the realization of the plan of prophylactic measures in the area.

Method of Carrying Out the Exercise. The instructor mentions to the students the technique of injecting vaccines against anthrax. The students are divided into groups of four; they examine the animals and take their temperatures. After this, under the direct observation of the instructor, the students set about vaccinating the animals. In each group the duties are divided: one student prepares the injection area; two hold down the animals, and one injects the vaccines. During the work the students change places.

After giving the inoculations the students make out a list of animals and a document on the inoculations given.

Inoculations against Anthrax

Inoculations against anthrax may be prophylactic or compulsory.

Protective (prophylactic) inoculations are given each year in the spring or autumn to all susceptible animals in fixed places which are endangered by disease or unfavorable with respect to disease. Non-inoculated animals newly brought into these areas must also be

vaccinated before being allowed into the general herd.

Inoculations against anthrax are permitted only by veterinary physicians or veterinary assistants. It is compulsory accurately to record the inoculated animals with a reading of the results of the inoculation. For this purpose a list is made up of the inoculated animals (see Exercise 14 for the form of the list), where the nature of complications is also indicated if they are observed.

For protective inoculations the following vaccines are used: Tsenkovskiy's first and second anthrax vaccines, STI anthrax vaccine, aluminum-hydroxide precipitated vaccine of the GNKI against anthrax.

Immunity in the animals occurs 10 days after inoculation with these vaccines and lasts 12 months.

Compulsory inoculations are given when anthrax appears in farms which are unfavorable with respect to the disease or in inhabited places or in an endangered area. The compulsory inoculations are of different character.

a) Inoculation of anthrax serum with subsequent injection of vaccine (done only for animals which have been in direct contact with sick animals or animals which died); b) Simultaneous injection of anthrax antiserum and Tsenkovskiy's second vaccine; c) Inoculation of vaccine alone.

Before the inoculations a veterinary examination is made of the animals and, if necessary, their temperatures are taken. Animals in farms in which there are other acute infectious diseases as well as animals with elevated temperatures, in the last one-two months of gravidity, weak or debilitated animals, should not be inoculated with vaccines. Based on the epizootic situation these animals are given anthrax antiserum.

The vaccines should be injected into horses, cattle, deer and camels subcutaneously in the area of the middle third of the neck; into sheep, hogs and goats, into the hairless part of the inner surface of the thigh; into rams, goats and barrows, on the inner surface of the foreleg. The hair at the injection site of the vaccine is shaved off, and the injection area is treated with 70 percent alcohol or three percent phenol solution.

For the inoculations two-cc syringes with begunka [?, the word ordinarily means runners or rollers] are used. A plunger and needle should be well fitted to the syringe. All instruments are sterilized by boiling for 30 minutes before inoculations, during the inoculations and after completion of the work.

Each animal is inoculated with a separate sterile needle.

One or two days after the inoculation of the vaccines a reaction is observed in the animals which is characterized by a slight temperature elevation and slight swelling at the injection site. These signs disappear after four-five days. Sometimes, complications can occur, expressed in the occurrence of edema, marked temperature elevation, general severe condition. The animals can die unless appropriate measures are taken (application of cold to the edema site and the injection of anthrax antiserum in therapeutic doses; when necessary, cardiac agents).

Veterinary observation of the inoculated animals is conducted until the reaction to inoculation is completed; as a rule, this is 10 days. After this, the document on the inoculations given is made up (see Exercise 14 for the form of the document).

Tsenkovskiy's First and Second Anthrax Vaccines. Animals of all species except goats can be inoculated once with Tsenkovskiy's second vaccine in appropriate doses.

It is forbidden to inoculate young animals with vaccines until the indicated age (when necessary, they are inoculated with serum).

[The title of the Table at the top of page 154 reads as follows: Doses of Tsenkovskiy's Second Vaccine; the headings are: species of animals, age, dose of second vaccine (in cc). The terms in the first column, reading down, are the following: cattle, horses, camels, deer sheep, swine. The terms in the second column, reading down, are the following: from three months to one year, one year and over; from three months to two years; two years and over; under two years; over two years; of all ages; three months and over; two months to one year; over one year.]

Goats are inoculated twice beginning with the age of three months: first, with the first vaccine in a dose of 0.3 cc; then, after 10-12 days with the second vaccine in a dose of 0.1 cc.

With the exception of deer, the vaccine is given to all animals subcutaneously; deer are inoculated intradermally. In the case of combined (simultaneous) inoculations the animals are first given anthrax antiserum, and then Tsenkovskiy's second vaccine (on the other side of the animal's body).

The STI Anthrax Vaccine. This vaccine is used subcutaneously once in the following doses (expressed in cc):

[Near the bottom of page 154 the Table has the following headings, reading across, species of animals; age of animals. The latter column is divided into two parts: from two months to one year; over one year. The first column, reading down, has the following terms: cattle, horses, swine, camels.]

Deer of all ages are inoculated with vaccine in a dose of one cc; sheep over four months of age, in a dose of 0.25 cc. It is forbidden to inoculate young agricultural animals with STI vaccine until the age indicated, and the same applies to goats of all ages.

GNKI Aluminum-Hydroxide Precipitated Vaccine Against Anthrax. The vaccine is used subcutaneously, once, in the following doses (expressed in cc):

[The Table near the top of page 155 has the following headings: species of animals; age of animals. The latter is divided into two columns: from two months to one year; over one year. The entries in the first column, reading down, are: horses, cattle, sheep, swine, goats, deer, camels.]

Anthrax Antiserum. Serum is used for protective and therapeutic purposes as well as in simultaneous inoculations with Tsenkovskiy's second vaccine. The serum is injected subcutaneously in the following doses (expressed in cc):

[The Table near the bottom of page 155 has the following headings: for protective purposes, for therapeutic purposes, in simultaneous inoculations. The entries in the first column, reading down, are: horses and camels; adult cattle; sheep, goats, calves and swine. The first two entries in the third column are: 100 or more.]

In animals inoculated with serum immunity lasts 14 days. Serum may be injected intravenously for therapeutic purposes; it is recommended that cattle be injected intraperitoneally in the area of the "jejunal fossa."

Exercise 24

Anaerobic Infections (Bradzot, Tetanus, Necrobacillosis). Technique of Laboratory Examination

Assignment. 1. To become acquainted with the technique of the laboratory examination of material for anaerobic infections. Demonstration of nutrient media for the cultivation of anaerobes and methods of culture. To become acquainted with the method of cultivation of anaerobes (methods of creating anaerobic conditions). 2. To prepare and stain smears from cultures or to examine prepared smears under the microscope. 3. To prepare an emulsion from the organs of a dead body and make a culture on liquid nutrient media for the purpose of cultivating anaerobes.

Basic Equipment and Material. Prepared nutrient media: Martin's bouillon, Kitt-Tarozzi medium, meat infusion gelatin, meat infusion blood agar. Culture plates containing agar, loops, pipets, glass marking pencils, alcohol burners, dryer, Komosovskiy pump, incubator, container with disinfectant solution, sterile mortars and pestles, sterile physiological saline, water baths, spatula, sets of stains for Gram staining and for staining for spores, cedar oil, and microscopes. A culture of the pathogens of necrobacillosis, tetanus or bradzot [malignant edema]. The organs of an animal which died of a noncontagious disease.

Place of the Exercise. The laboratory of the technical school.

Method of Performing the Exercise. The instructor acquaints the students with the technique of laboratory examination of pathological material for anaerobic infections, shows the most frequently used nutrient media for the cultivation of anaerobes, demonstrates methods of subculturing anaerobic cultures from one medium on another. Then, the instructor shows how anaerobic conditions are created for the growth of cultures, and demonstrates apparatus (dryer, pumps, and others).

The students independently prepare smears from the cultures of the pathogens of necrobacillosis, tetanus or bradzot and stain them for spores and by the Gram method. In the absence of these cultures ready-made smears are used. The students draw the microscope picture observed. Then, an emulsion is made from the organs of the animal (which died of a noncontagious disease), and cultures are made on liquid nutrient media for the purpose of cultivating

anaerobes.

Laboratory Examination of Material for Anaerobic Infection. It is necessary to send the following pathological material for laboratory examination.

For bradsot: an exudate from the inflammatory edema in sealed pipets and smear-impressions from affected muscles on glass slides. Pieces of parenchymatous organs and affected muscles are sent from the dead animals.

For tetanus: material from the wound.

For necrobacillosis: necrotic areas from the affected organs and tissues along with the adjacent healthy tissue and smears taken from the involved tissue next to the healthy tissue.

In the laboratory the following are done: 1) microscopic examination of the smears (for the preparation of smears see Exercise 3); 2) cultures on nutrient media (see Exercise 7); 3) infection of laboratory animals (see Exercise 9).

For the original isolation of the anaerobic microbes from the material investigated most often use is made of liquid nutrient media: Martin's bouillon, Kitt-Tarozzi medium, which contain pieces of animal tissue (liver, kidney, spleen). The media are poured out into test tubes forming a high column. Vaseline or paraffin oil (one-two cc) are layered onto the surface of the bouillon. Before making the culture the medium is heated for 20-30 minutes in a boiling water bath in order to remove the air which has entered the bouillon during the standing of the medium. Then the test tubes are rapidly cooled and a culture is made. The culture material is introduced into the medium with a pipet or loop.

For the cultivation of anaerobes use is also made of meat infusion gelatin (the culture is made by puncture; see Exercise 7), semiliquid agar containing glucose, brain, blood and serum media.

Emulsions from pieces of internal organs and affected muscles serve as the material for culture in the examination for the presence of anaerobic microflora. Before the preparation of the emulsions mortars together with pestles wrapped in paper are sterilized in a dryer or in an autoclave. The place from which the material is taken is first burned or cauterized with a heated spatula. A piece of the organ or muscle is cut out with a sterile scalpel or scissors, placed in the mortar and carefully ground up with the pestle. The emulsion is prepared in sterile physiological saline; one part of material and nine parts of physiological saline (by volume) are taken.

The material from the prepared emulsion is used to make

cultures on nutrient media and infect laboratory animals (when necessary). Simultaneously, for a check on the presence of anaerobic microflora the same material is plated out on simple nutrient media meat infusion agar and meat infusion bouillon.

The cultures on special liquid media (Martin's bouillon, Kitt-Tarozzi medium) are cultivated in an incubator under ordinary aerobic conditions. The cultures on meat infusion gelatin are kept for five-six days at room temperature. The cultures are grown out on solid media under anaerobic conditions.

The simplest method of creating anaerobic conditions is that of pumping out the air from the dryer in which the media containing the anaerobe cultures have been placed. For this purpose metal microanaerostats [apparently, micro-pressure gages] are used. The air is pumped out by means of pumps (oil pumps, Komosovskiy pump and others). Anaerobic conditions may be obtained by burning alcohol in a dryer.

After pumping the air out the dryers are placed in an incubator. The results of the growth are read after one-two days. For this purpose smears are made from the cultures with a pipet or loop. In the absence of growth the cultures are kept in the incubator another four-six days.

Exercise 30

Brucellosis. Allergic Diagnosis of the Disease and Vaccination of Animals

Assignment 1. To master the technique of injecting brucellosis allergens into different agricultural animals. 2. To study methods of evaluating the reaction. 3. To master the technique of dilution and application of the brucellosis vaccine. To mark the vaccinated animals.

Basic Equipment and Material. Allergens: abortin, brutsellizat, brutsellogidrolizat. Dry brucellosis vaccine from strain No 19. Instructions for the use of these biologicals. Two-five-cc syringes, needles for the syringes, sterilizer, forceps, scissors, Primus or electric stove, cotton, three percent phenol solution and sterile physiological saline. Sterile bottles for the dilution of the dry brucellosis vaccine. Branding irons for marking animals inoculated with the brucellosis vaccine.

Animals: calves (under a year), sheep, goats, swine.

Place of the Exercise. Animal husbandry farm, meat combine. At practical exercises during school practice this exercise should be conducted on animals on which it is planned to make an examination for brucellosis. In this case, the entire investigation plan is carried out (for example: the double intradermal test with brutsellizat in sheep with reading of the reaction after 48-72 hours).

Method of Carrying Out the Exercise. The instructor mentions to the students the methods of injecting brucellosis allergens as well as the technique of dilution and utilization of dry brucellosis vaccine from strain No 19. Positive and doubtful allergic tests on animals or on tables (placards) should be shown.

The students are divided into groups of four: one prepares the injection area, another injects the preparation, and two hold down the animal. During the course of the work the students change places. Instead of allergic preparations sterile physiological saline can be given for this work.

The instructor checks the degree to which methods of administering the allergens of brucellosis vaccine have been mastered by every student.

The Use of Abortin for the Diagnosis of Brucellosis in Young Cattle. Abortin is used for allergic diagnosis of brucellosis in young

cattle under a year of age. Before using, the preparation is carefully shaken up. The instruments are sterilized by boiling before work. The upper end of the ampule containing the preparation is flamed before breaking off. Disinfection of the injection area is carried out with alcohol or three percent phenol.

Abortin is injected intradermally in the middle of one of the subcaudal folds in a dose of 0.2 cc. The other fold is used for control and comparison.

The assistant holds the tail to the side. The subcaudal fold is taken in the left hand and the needle is injected strictly intradermally, holding the syringe almost parallel to the skin fold. With proper intradermal injection of abortin a bump the size of a pea forms at the injection site. A separate sterile needle (Nos 28-32) is used for each animal.

The reactions are read two times, at 48 ad 72 hours (Fig 76), by means of feeling the injection site and comparing this fold with the control.

[Fig 76. Diagram of the Use of Abortin in Calves. Reading the legend in the boxes clockwise from 12:00 o'clock, the entries are as follows: those reacting positively are isolated; those reacting doubtfully are isolated until the next examination; second reading of the reaction; first reading of the reaction; injection of abortin. The words seen in the middle following the numbers are "hours"].

Positive reaction: edema at the injection site of abortin, usually with thickening in the middle.

Doubtful reaction: poorly-expressed edema, which is determined only by palpation and by comparison with the other subcaudal fold; or else a thickening the size of a pea without pronounced edema forms at the injection site.

Negative reaction: no changes are observed at the injection site.

The animals in which doubtful reaction is noted are isolated and examined again after a month; thereby, abortin is injected into the other subcaudal fold. Animals which show a doubtful reaction twice are considered positive.

The Use of Brutsellizat for the Diagnosis of Brucellosis in Sheep and Goats. For the allergic diagnosis of brucellosis in sheep and goats brutsellizat is injected intradermally (Fig 77) into one of the subcaudal folds in a dose of 0.2 cc with a fine needle. The other

fold serves as a comparison in reading the reaction. The reaction is read by means of examination and palpation of the injection site in comparison with the control fold.

Positive reaction: well-expressed edema at the injection site.

Doubtful reaction: well-expressed edema, which is found only on palpation and comparison with the control fold.

Negative reaction: no changes noted at the injection site.

Fig 77. Injection of Allergen Into the Subcaudal Fold: a. Subcaudal folds.

Animals in which a positive reaction is found are isolated. Animals in which a doubtful or negative reaction is recorded on the first reading (after 48 hours) are again injected with the brutsellizat in the same place and in the same dose. After the second injection the reaction is read after 24 hours (Fig 78).

[Fig 78. Diagram of Utilization of Brutsellizat in Sheep. Reading clockwise from 12:00 o'clock, those reacting positively are isolated; those reacting doubtfully are isolated until the next examination; second reading of the reaction; first reading of the reaction and second injection of brutsellizat into negatively and doubtfully reacting animals; first injection of brutsellizat].

The Use of Brutsellogidrolizat for the Diagnosis of Brucellosis in Sheep, Goats and Swine. The use of brutsellogidrolizat in sheep and goats is absolutely the same as the utilization of brutsellizat described above.

Swine are injected intradermally with brutsellogidrolizat on the outside of the ear at its base. At the injection site of the preparation an inflammatory reaction is noted in swine sick with brucellosis (swelling, hyperemia, sometimes hemorrhage in the form of a dark red spot in the center of the edema) -- a positive reaction.

The doubtful reaction is characterized by poorly-expressed edema.

In the absence of changes at the injection site the reaction is read as negative.

The reaction is read twice in swine: after 24 and 48 hours (Fig 79).

[Fig 79. Diagram of Use of Brutsellogidrolizat in Swine. Reading

clockwise from 12:00 o'clock, those reacting positively are isolated; those reacting doubtfully are isolated until the next examination; second reading of the reaction; first reading of the reaction; injection of brutsellogidrolizat].

All swine showing a positive reaction are immediately isolated. Swine with a doubtful reaction are isolated and kept as a separate group; after a month they are again examined with brutsellogidrolizat in the same manner.

The Use of Dry Brucellosis Vaccine Made of Strain No 19. Rules for Diluting the Vaccine. The neck of the ampule is wiped with alcohol and broken off. With a sterile syringe and needle three-five cc of sterile physiological saline are introduced into the ampule. The ampule is carefully shaken until a uniform suspension is obtained. The suspension is sucked up by the syringe and needle and transferred to a sterile bottle. In this bottle the required quantity of sterile physiological solution is poured for the dilution of the dry vaccine.

For subcutaneous injection of cattle the dose of vaccine is diluted in five cc of physiological saline and injected into the middle third of the neck.

The vaccine may be injected intradermally also. For this purpose the dose of vaccine is dissolved in 2.5 cc of physiological saline and injected into each animal in a dose of 0.5 cc of the dilute vaccine (one-fifth of the dose for subcutaneous injection) intradermally into the subcaudal fold.

For the vaccination of short-horned cattle the dose of dry vaccine used for long-horned cattle is diluted in four cc of physiological saline. Each animal is given two cc of vaccine diluted in this way subcutaneously in the region of the elbow, inner surface of the thigh or tail.

All animals vaccinated with dry brucellosis vaccine made of strain No 19 are marked by means of drilling a round hole into the base of the left ear.

Exercise 35

Foot-and-Mouth Disease. Vaccination of Animals against Foot-and-Mouth Disease

Assignment. To master methods of vaccinating animals against foot-and-mouth disease.

Basic Equipment and Material. VIEV [All-Union Institute of Experimental Veterinary Medicine] foot-and-mouth vaccine. Instructions on the use of the vaccine. Sterile physiological saline. Two-five-cc syringes, needles for the syringes, sterilizer, scissors, forceps, Primus or electric stove, three percent phenol solution and cotton. Instruments for holding the animals down.

Animals: cattle, swine, sheep and goats.

Place of the Exercise. The exercise is held in an animal husbandry farm during school practice or in the clinic of the technical school.

Method of Conducting the Exercise. The instructor acquaints the students with the method of using foot-and-mouth disease vaccine, demonstrates the injection of the vaccine intradermally and submucosally into the lip.

The students are divided into groups of four and the duties are divided among them: two hold the animal down, a third prepares the injection area, the fourth injects the vaccine. During the work the students change places. In conducting the exercise on the animals of the technical school vivarium the students are given sterile physiological saline instead of vaccine. The instructor checks on and grades the work of each student.

Inoculation of Animals with the VIEV Foot-and-Mouth Vaccine. The vaccine is used for the purpose of prophylaxis of foot-and-mouth disease in cattle, swine, sheep and goats. Before using, the vaccine is carefully shaken up. The vaccine is injected once. The hair at the injection site of the vaccine is shaved off; the skin is disinfected with three percent phenol solution. The syringes and needles are sterilized by boiling.

Methods of Using the Vaccine: in long-horned cattle, subcutaneously or intradermally (at the base of the ear) or submucosally in the upper lip; in swine, subcutaneously in the area of the inner surface of the thigh or at the base of the ear; in sheep and goats, subcutaneously in the area of the internal surface of the thigh, intrader-

mally into the subcaudal fold or submucosally into the lip.

[The Table at the bottom of page 198 is entitled Doses of Vaccine (in cc). The headings read: species of animals, subcutaneously, intradermally, submucosally into the lip. The entries in the first column, reading down, are: long-horned cattle: over six months of age, under six months of age; swine over eight months of age; young pigs from the fourth to the eighth month of age; suckling pigs and those which have just been weaned; adult sheep and goats; suckling lambs and goats.] .

The methods of injecting the biologicals subcutaneously and intradermally are described in Exercise 14.

In order to inject the vaccine submucosally into the upper lip it is convenient to use the following technique. The one holding down the animal takes the cow by the horns and with a quick movement turns the cow on its side. For a certain time the cow offers no resistance. The operator quickly draws out the upper lip of the animal with the left hand and by means of the syringe and needle injects the vaccine into the submucosa of the upper lip with the right hand (into an area without papillae).

Exercise 46

Infectious Diseases of Swine

Diagnosis and Differential Diagnosis of the Main Infectious Diseases of Swine

Assignment. To gain skills in the diagnosis and differential diagnosis of the main infectious diseases of swine.

Basic Equipment and Material (see Exercise 47). Guide books, textbooks on epizootology.

Place of the Exercise. Animal husbandry farm, lecture-room of the technical school.

Method of Conducting the Exercise. The study of this section is advisably conducted on a swine-raising farm. Before going to the farm the students should independently make up tables of differential diagnosis of the main infectious diseases of swine, which will contribute to mastering the principles on which the diagnosis of one disease or another is based. With this aim in view the instructor gives the students a diagram (page 31), from which the table should be made up; he indicates which disease signs should attract attention in the differentiation.

Table of Differential Diagnosis of the Most Important Infectious Diseases of Swine (Schema)

(As an Example the Data on Swine Influenza have been Filled in)

[The main headings of the Table are: signs for differentiation, diseases. The latter heading is subdivided into the following: plague, erysipelas, Aujeszky's disease, pasteurellosis, listerellosis, paratyphoid, influenza. The rest of the Table is shown below:]

Disease pathogen	Virus and hemophilic microbe
Epizootological Differentiation	
Course of the infectious disease (epizootic, sporadic cases)	Enzootic or epizootic; Rapid inclusion of the whole group; high degree of infectivity

Age of the sick animals	Swine of all ages become sick
Predisposing factors	Cooling of the organism (cold)
Seasonality	In cold and rainy seasons
Sources of infection and routes of infection	Sick virus carriers. Chiefly by the aerogenic route
Clinical Differentiation	
Forms and duration of the disease	Acute septicemic with pneumonia; duration, seven-10 days
Body temperature and nature of the fever	High body temperature, fever of continuous type
Skin changes	Absent
Changes in the Visible Mucosae	Conjunctivitis, nose-bleeding
Condition of the Respiratory Organs	Cough, sneezing, labored breathing. Signs of bronchopneumonia.
Condition of digestive organs	No appetite
Pathological Differentiation	
Condition of serous and mucous membranes (presence of hemorrhages)	Changes are not characteristic
Changes in the lymph nodes	The same
Changes in the liver and spleen	The same

Changes in the gastrointestinal tract	Same
Changes in the respiratory organs	Bronchopneumonia, fibrinous pneumonia pleurisy

Differentiation by Means of Specific Agents and Antibiotics

Use of specific sera	Not worked out
Use of antibiotics (penicillin)	Not used

Laboratory Examination

Pathogen found (bacteriological and virological examinations)	B. hemophilus influenzae suis isolated

Students making use of guide and textbooks independently make up a table. Then, the instructor makes an analysis of the tables made. A home assignment may be given, and the tables made out can be analyzed at the class exercises.

In the farm the exercise is conducted directly on sick animals. Under the direction of the instructor the students examine the animals clinically (see Exercise 1), study the epizootological data and the conditions influencing the occurrence and spread of infectious diseases in swine, take material for laboratory examination. If there are dead bodies of animals available they are subjected to pathological study. In making the diagnosis the instructor should draw attention to differentiation of infectious diseases from noncontagious swine diseases.

When the exercises are held in an animal husbandry farm the instructor can combine topics 46 and 47 and first examine the veterinary-sanitary condition of the farm and then make a clinical examination of sick animals, analyze the epizootic situation, outline a plan of measures for the elimination of the disease and organize the giving of inoculations, use therapeutic agents (antibiotics and others).

Exercise 47

The Use of Biologicals and Antibiotics for Infectious Diseases of Swine

Assignment. 1. To become acquainted with biologicals used for the main infectious diseases of swine. 2. To become acquainted with the organization of inoculations of swine. To master the technique of injecting swine with different biologicals and antibiotics. To become acquainted with the giving of simultaneous inoculations.

Basic Equipment and Materials. Biologicals: glycerinated crystal-violet vaccine against hog cholera, dry lapinized avirulent virus-vaccine against hog cholera (ASV), aluminum-hydroxide precipitated formol vaccine against bacillary swine erysipelas, long-acting vaccine against swine erysipelas, aluminum-hydroxide precipitated vaccine against Aujeszky's disease, sera against swine cholera and erysipelas. Instructions for the use of these biologicals. Antibiotics -- penicillin. Two-, five-, 10-, 20-cc syringes, needles for the syringes, sterilizer, forceps, scalpels, scissors, electric stove or Primus, sterile physiological saline, cotton, three percent phenol solution, glass rods, a system with an Agali cock. Stethoscopes, thermometers, towel, soap, disinfectant solution for treating the hands. Instruments for holding down the swine, containers for taking pathological material from animals which are sick and have died (jars, test tubes). Animals: swine.

Place of the Exercise. Swine-raising farm or meat combine.

Method of Conducting the Exercise. The instructor shows different methods of injecting biologicals on animals. Then the students are divided into groups of four; the duties are divided among them and the students independently master the technique of injecting biologicals and antibiotics by different methods. During the work the students change places within the group. The instructor checks on the work of the students.

Instead of biologicals and antibiotics sterile physiological saline can be given for injecting the animals.

Note. Organization of inoculations in swine, methods of holding them down and methods of injecting the biologicals into swine have been described in Exercise 14.

Glycerinated Crystal-Violet Vaccine Against Hog Cholera. The vaccine is used for active immunization of swine in inhabited places (or farms) which are unfavorable with respect to hog cholera and those

which are endangered by it. In giving the inoculations the established rules of asepsis and and antisepsis are observed; the instruments are sterilized by boiling. A separate sterile needle is used for each animal.

The vaccine is injected by two methods: subcutaneously or intradermally.

The vaccine is injected subcutaneously into the base of the ear or into the inner surface of the thigh twice at an interval of 10-14 days, in doses of five cc each, into swine of all ages (beginning with two months).

The vaccine is injected intradermally into the base of the ear, on the outside, two times with an interval of seven-eight days, in doses of one cc, into swine of all ages (beginning with two months).

Gravid sows are not inoculated 14 days before farrowing and less than 10 days after farrowing. Swine inoculated with glycerinated crystal-violet vaccine are kept under veterinary observation for three-four weeks.

Dry Lapinized Virus-Vaccine Against Hog Cholera (ASV). Used in farms which are unfavorable with respect to the cholera and which are directly endangered.

Before use, the dry virus-vaccine is diluted in sterile physiological saline. Per cc of vaccine mass 50-100 cc of sterile physiological saline are used. The ampule is opened, thereby observing rules of asepsis. With a sterile glass or metal rod the ampule contents are ground up into a powder and then physiological saline solution is introduced with a sterile syringe. The ampule is covered with cotton and, shaking it periodically, it is allowed to stand for 10-15 minutes. After the ampule contents have dissolved they are poured into a bottle containing sterile physiological saline. The diluted vaccine is injected intramuscularly in a dose of two cc, regardless of the age or weight of the swine.

The use of the dry virus-vaccine is permitted simultaneously with serum against hog cholera (simultaneous inoculations). Thereby, the serum is injected directly before injection of the vaccine or simultaneously with it, subcutaneously, on the other side of the body in appropriate doses.

Aluminum-Hydroxide Precipitated Formol Vaccine Against Bacillary Swine Erysipelas. Used for protective and compulsory inoculations of swine against erysipelas.

All swine beginning with the age of two months are vaccinated (gravid sows are vaccinated no sooner than two days after or no less

than five days before farrowing). The vaccine is injected intramuscularly, twice, with a 12-14-day interval, in a dose of five cc each time, regardless of the weight or age of the animal.

Long-Acting Vaccine Against Swine Erysipelas. It is used for the prophylaxis of swine erysipelas as well as in farms where this disease has occurred. All swine, beginning with the age of two months, are vaccinated, with the exception of suckling and gravid sows within a month before farrowing and no sooner than a month after farrowing.

The vaccine is injected subcutaneously on the inside of the thigh or in the neck region at the base of the ear. The animals are vaccinated twice, with a 12-14-day interval between inoculations; the dose of the first inoculation is 0.3 cc; of the second, 0.5 cc.

Aluminum-Hydroxide-Precipitated Formol Vaccine Against Aujeszky's Disease. It is used in farms which are unfavorable for Aujeszky's disease or endangered by it. The vaccine is injected intramuscularly, twice, with a six-eight-day interval between inoculations.

[The Table at the top of page 236 is entitled Doses of Vaccines (in cc). Its three headings are: species of animals, the first time, the second time. The entries in the first column, reading down, are: suckling pigs from the age of 10 days to 45 days; suckling pigs older than 45 days and those which have been weaned and are under four months of age; young and adult pigs.]

Serum Against Hog Cholera. The serum is injected subcutaneously at the base of the ear or on the inside of the thigh for prophylactic purposes and in simultaneous inoculations in the following doses:

Suckling pigs	15 cc
Weaned baby pigs.	15-20 cc
Young pigs	20-30 cc

Adult swine are inoculated according to the calculation of one cc per kg of weight.

The serum is injected in double the dose for therapeutic purposes. For better absorption no more than 50 cc of serum should be injected into one place.

Serum Against Swine Erysipelas. The serum is injected for therapeutic and prophylactic purposes subcutaneously or intramuscularly.

[The Table near the bottom of page 236 is entitled Doses of Serum (in cc). Its two headings are: for therapeutic purposes; for prophylactic purposes. Reading down in the first column we have: suckling pigs; young pigs weighing under 50 kilograms; pigs weighing more than 50 kilograms.]

The Use of Penicillin for Swine Erysipelas. Penicillin is used in swine sick with erysipelas, those suspected of the disease and those which have complications after vaccination.

The preparation is injected intramuscularly in a dose of 2,000-3,000 units per kg of the animal's weight two-three times at eight-12-hour intervals. After the temperature drops, one-two injections more should be given for the prevention of recurrences of the disease.

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